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(54) Titre : COMPOSITIONS ET PROCEDES POUR CORRIGER DES MUTATIONS DE LA DYSTROPHINE DANS DES CARDIOMYOCYTES HUMAINS

(54) Title: COMPOSITIONS AND METHODS FOR CORRECTING DYSTROPHIN MUTATIONS IN HUMAN CARDIOMYOCYTES

(57) Abrégé/Abstract:
The disclosure provides a method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising administering to the subject a Cas9 nuclease or a sequence encoding a Cas9 nuclease, and a gRNA or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene. The administering restores dystrophin expression in at least a subset of the subject's cardiomyocytes, and may at least partially or fully restore cardiac contractility.

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(54) **Title:** COMPOSITIONS AND METHODS FOR CORRECTING DYSTROPHIN MUTATIONS IN HUMAN CARDIOMYOCYTES

(57) **Abstract:** The disclosure provides a method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising administering to the subject a Cas9 nuclease or a sequence encoding a Cas9 nuclease, and a gRNA or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene. The administering restores dystrophin expression in at least a subset of the subject's cardiomyocytes, and may at least partially or fully restore cardiac contractility.

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COMPOSITIONS AND METHODS FOR CORRECTING DYSTROPHIN MUTATIONS IN HUMAN CARDIOMYOCYTES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 62/624,748, filed January 31, 2018, which is incorporated by reference herein in its entirety for all purposes.

FEDERAL FUNDING SUPPORT CLAUSE

This invention was made with government support under grants no. HL-130253, HL-077439, DK-099653, and AR-067294 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 31, 2019, is named UTFDP0002WO.txt and is 1,722,119 bytes in size.

FIELD OF THE DISCLOSURE

The present disclosure relates to the fields of molecular biology, medicine and genetics. More particularly, the disclosure relates to compositions and uses thereof for genome editing to correct mutations *in vivo* using an exon-skipping approach.

BACKGROUND

Muscular dystrophies (MD) are a group of more than 30 genetic diseases characterized by progressive weakness and degeneration of the skeletal muscles that control movement. Duchenne muscular dystrophy (DMD) is one of the most severe forms of MD that affects approximately 1 in 5000 boys and is characterized by progressive muscle weakness and premature death. Cardiomyopathy and heart failure are common, incurable and lethal features of DMD. The disease is caused by mutations in the gene encoding dystrophin (*DMD*), a large intracellular protein that links the dystroglycan complex at the cell surface with the underlying cytoskeleton, thereby maintaining integrity of the muscle cell membrane during contraction. Mutations in the dystrophin gene result in loss of expression of dystrophin, causing muscle membrane fragility and progressive muscle wasting.

SUMMARY

Genomic editing with CRISPR/Cas9 is a promising new approach for correcting or mitigating disease-causing mutations. Duchenne muscular dystrophy (DMD) is associated with lethal degeneration of cardiac and skeletal muscle caused by more than 3000 different mutations in the X-linked dystrophin gene (DMD). Most of these mutations are clustered in "hotspots." As described in the Examples herein, a screen was performed for optimal guide RNAs capable of introducing insertion/deletion (indel) mutations by nonhomologous end joining that abolish conserved RNA splice sites in 12 exons that potentially allow skipping of the most common mutant or out-of-frame DMD exons within or nearby mutational hotspots. The correction of DMD mutations by exon skipping is referred to herein as "myoediting." In proof-of-concept studies, myoediting was performed in representative induced pluripotent stem cells from multiple patients with large deletions, point mutations, or duplications within the DMD gene and efficiently restored dystrophin protein expression in derivative cardiomyocytes. In three-dimensional engineered heart muscle (EHM), myoediting of DMD mutations restored dystrophin expression and the corresponding mechanical force of contraction. Correcting only a subset of cardiomyocytes (30 to 50%) was sufficient to rescue the mutant EHM phenotype to near-normal control levels. Thus, it is shown that abolishing conserved RNA splicing acceptor/donor sites and directing the splicing machinery to skip mutant or out-of-frame exons through myoediting allows correction of the cardiac abnormalities associated with DMD by eliminating the underlying genetic basis of the disease.

Thus, in some embodiments, the disclosure provides a method for editing a mutant dystrophin gene in a cardiomyocyte, the method comprising contacting the cardiomyocyte with a Cas9 nuclease or a sequence encoding a Cas9 nuclease, and a gRNA or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene.

The disclosure also provides a method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising administering to the subject a Cas9 nuclease or a sequence encoding a Cas9 nuclease, and a gRNA or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene; wherein the administering restores dystrophin expression in at least 10% of the subject's cardiomyocytes.

The disclosure also provides a method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising contacting an induced

pluripotent stem cell (iPSC) with a Cas9 nuclease or a sequence encoding a Cas9 nuclease, and a gRNA, or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene; differentiating the iPSC into a cardiomyocyte; and administering the cardiomyocyte to a the subject.

Also provided is a cell (such as an induced pluripotent stem cell (iPSC) or cardiomyocyte) produced according to the methods of the disclosure, and compositions thereof. In some embodiments, the cell expresses a dystrophin protein.

Also provided is an induced pluripotent stem cell (iPSC) comprising a Cas9 nuclease, or a sequence encoding a Cas9 nuclease, and a gRNA, or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene.

As used in the specification, "a" or "an" may mean one or more. As used in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one.

The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, for the method being employed to determine the value, or that exists among the study subjects. Such an inherent variation may be a variation of ±10% of the stated value.

Throughout this application, nucleotide sequences are listed in the 5' to 3' direction, and amino acid sequences are listed in the N-terminal to C-terminal direction, unless indicated otherwise.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1A-1C. Myoediting strategy and identification of optimal guide RNAs to target the top 12 exons in DMD. (FIG. 1A) Conserved splice sites contain multiple NAG and NGG sequences, which enable cleavage by SpCas9. The numbers indicate the frequency of occurrence (%). (FIG. 1B) Human DMD exon structure. Shapes of intron-exon junctions indicate complementarity that maintains the open reading frame upon splicing. Red arrowheads indicate the top 12 targeted exons. The numbers indicate the order of the exons. (FIG. 1C) T7E1 assays in human 293 cells transfected with plasmids expressing the corresponding guide RNA (gRNA), SpCas9, and GFP for the top 12 exons. The PCR products from GFP+ and GFP− cells were cut with T7 endonuclease I (T7E1), which is specific to heteroduplex DNA caused by CRISPR/Cas9-mediated genome editing. Red arrowhead indicates cleavage bands of T7E1. M denotes size marker lane. bp indicates the base pair length of the marker bands.

FIG. 2A-2J. Rescue of dystrophin mRNA expression in iPSC-derived cardiomyocytes with diverse mutations by myoediting. (FIG. 2A) Schematic of the myoediting of DMD iPSCs and 3D-EHMs–based functional assay. (FIG. 2B) Myoediting targets the exon 51 splice acceptor site in Del DMD iPSCs. A deletion (exons 48 to 50) in a DMD patient creates a frameshift mutation in exon 51. The red box indicates out-of-frame exon 51 with a stop codon. Destruction of the exon 51 splice acceptor in DMD iPSCs allows splicing from exons 47 to 52 and restoration of the dystrophin open reading frame. (FIG. 2C) Using the guide RNA library, three guide RNAs (Ex51-g1, Ex51-g2, and Ex51-g3) that target sequences 5′ of exon 51 were selected. FIG. 2C discloses SEQ ID NO: 2481. (FIG. 2D) RT-PCR of cardiomyocytes differentiated from uncorrected DMD (Del), corrected DMD iPSCs (Del-Cor.), and WT. Skipping of exon 51 allows splicing from exons 47 to 52 (lower band) and restoration of the DMD open reading frame. (FIG. 2E) Myoediting strategy for pseudo-exon 47A (pEx). DMD exons are represented as blue boxes. Pseudo-exon 47A (red) with stop codon is marked by a stop sign. The black box indicates myoediting-mediated indel. (FIG. 2F) Sequence of guide RNAs for pseudo-exon 47A of pEx. DMD exons are represented as blue boxes, and pseudo-exons are represented as red boxes (47A). sgRNA, single-guide RNA. FIG. 2F discloses SEQ ID NOS 2482-2484, respectively, in order of appearance. (FIG. 2G) RT-PCR

of human cardiomyocytes differentiated from WT, uncorrected DMD (pEx), and corrected DMD iPSCs (pEx-Cor.) by guide RNAs In47A-g1 and In47A-g2. Skipping of pseudo-exon 47A allows splicing from exons 47 to 48 (lower band) and restoration of the DMD open reading frame. (FIG. 2H) Myoediting strategy for the duplication (Dup) of exons 55 to 59. DMD exons are represented as blue boxes. Duplicated exons are represented as red boxes. The black box indicates myoediting-mediated indel. (FIG. 2I) Sequence of guide RNAs for intron 54 of Dup (In54-g1, In54-g2, and In54-g3). FIG. 2I discloses SEQ ID NOS 2485-2487, respectively, in order of appearance. (FIG. 2J) RT-PCR of human cardiomyocytes differentiated from WT, uncorrected DMD (Dup), and corrected DMD iPSCs (Dup-Cor.). Skipping of duplicated exons 55 to 59 allows splicing from exons 54 to 55 and restoration of the DMD open reading frame. RT-PCR of RNA was performed with the indicated sets of primers (F and R) (Table 4).

FIG. 3A-3F. Immunocytochemistry and Western blot analysis show dystrophin protein expression rescued by myoediting. (FIG. 3A to 3C) Immunocytochemistry of dystrophin expression (green) shows DMD iPSC cardiomyocytes lacking dystrophin expression. Following successful myoediting, the corrected DMD iPSC cardiomyocytes express dystrophin. Immunofluorescence (red) detects cardiac marker troponin-I. Nuclei are labeled by Hoechst dye (blue). (FIG. 3D to 3F) Western blot analysis of WT (100 and 50%), uncorrected (Del, pEx, and Dup) and corrected DMD (Del-Cor#27, pEx-Cor#19, and Dup-Cor#6.) iCM. Red arrowhead (above 250 kD) indicates the immunoreactive bands of dystrophin. Blue arrowhead (above 150 kD) indicates the immunoreactive bands of MyHC loading controls. kD indicates protein molecular weight. Scale bar, 100 mm.

FIG. 4A-4F. Rescued DMD cardiomyocyte-derived EHM showed enhanced FOC (force of contraction). (FIG. 4A) Experimental setup for EHM preparation, culture, and analysis of contractile function. (FIG. 4B to 4D) Contractile dysfunction in DMD EHM can be rescued by myoediting. FOC normalized to muscle content of each individual EHM in response to increasing extracellular calcium concentrations; n = 8/8/6/4/6/6/4/4; *P < 0.05 by two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. WT EHM data are pooled from parallel experiments with indicated DMD lines and applied to Fig. 4 (B to D). (FIG. 4E) Maximal cardiomyocyte FOC normalized to WT. n = 8/8/6/4/6/6/4/4; *P < 0.05 by one-way ANOVA and Tukey's multiple comparison test. (FIG. 4F) Titration of corrected cardiomyocytes revealed that 30% of cardio-myocytes needed to be repaired to partially rescue the phenotype, and 50% of cardiomyocytes needed to be repaired to fully rescue the phenotype (100% Del-Cor.) in EHMs. WT, Del, and 100% Del-Cor. are pooled data, as displayed in Fig. 4.

FIG. 5A-5B. Genome editing of DMD top 12 exons by CRISPR/Cas9. (FIG. 5A) DNA sequences of DMD top 12 exons (51, 45, 53, 44, 46, 52, 50, 43, 6, 7, 8 and 55) from GPF+ human 293 cells edited by SpCas9 using the corresponding guide RNAs (Table 5). PCR products from genomic DNA of each sample were subcloned into pCRII-TOPO vector and individual clones were picked and sequenced. Unedited wild type (WT) sequences are on the top and representative edited sequences are on the bottom. Deleted sequences are replaced by black dashes. Red lower case letters (ag) indicate the splice acceptor sites (SA, 3' end of the intron). Blue lower case letters (gt) indicate the splice donor sites (SD, 5' end of the intron). FIG. 5A discloses SEQ ID NOS 2488-2526 in the left column and SEQ ID NOS 2427-2546 in the right column, all respectively, in order of appearance. (FIG. 5B) RT-PCR of RNA from edited 293 cells indicate deletion of targeted DMD Dp140 isoform exons (51, 53, 46, 52, 50 and 55). Black arrows indicate the RT-PCR products with exon deletions. M denotes size marker lane. bp indicates the length of the marker bands. Sequence of the RT-PCR products of exon deletion bands contained the two flanking exons, but skipped the targeted exon. For example, sequence of the RT-PCR products of ΔEx51 band confirmed that exon 50 spliced directly to exon 52, excluding exon 51. FIG. 5B discloses "GAGCCTGCAACA" as SEQ ID NO: 2547, "ATCGAACAGTTG" as SEQ ID NO: 2548, "AAAGAGTTACTG" as SEQ ID NO: 2549, "CAGAAGTTGAAA" as SEQ ID NO: 2550, "GTGAAGCTCCTA" as SEQ ID NO: 2551 and "TAAAAGGACCTC" as SEQ ID NO: 2552.

FIG. 6A-6D. Correction of a large deletion mutation (Del. Ex47-50) in DMD iPSCs and iPSC-derived cardiomyocytes. (FIG. 6A) T7E1 assay using human 293 cells transfected with plasmid expressing SpCas9, gRNAs (Ex51-g1, g2 and g3), and GFP show genome cleavage at DMD exon 51. Red arrowheads point to cleavage products. M, marker; bp, base pair. (FIG. 6B) DNA sequences of DMD exon 51 from GPF+ DMD Del iPSCs edited by SpCas9 and the guide RNA Ex51 g3. PCR products from genomic DNA of a mixture of myoedited DMD iPSCs were subcloned into pCRII-TOPO vector and sequenced as described above. Uncorrected exon51 sequence is on the top and representative edited sequences are on the bottom. Deleted sequences are replaced by black dashes. Red lower-case letters (ag) indicate the splice acceptor sites. The number of deleted nucleotides is indicated by (-). FIG. 6B discloses SEQ ID NOS 2553-2561, respectively, in order of appearance. (FIG. 6C) Sequence of the lower RT-PCR band from Fig. 2D (Del-Cor. lane) confirms skipping of exon 51, which reframed the DMD ORF (dystrophin transcript from exons 47 to 52). FIG. 6C discloses SEQ ID NO: 2562. (FIG. 6D) Immunocytochemistry shows dystrophin expression in iPSC-derived cardiomyocyte mixtures (Del-Cor.) and single colony (Del-Cor-SC) following

SpCas9-mediated exon skipping with guide RNA Ex51-g3 compared to WT and uncorrected cardiomyocyte (Del). Green, dystrophin staining; red, troponin I staining; blue, nuclei staining. Scale bar = 100 μm.

FIG. 7A-7D. Correction of a pseudo-exon mutation (pEx47A) in DMD iPSCs and iPSC-derived cardiomyocytes. (FIG. 7A) T7E1 assay using DMD pEx47A iPSCs nucleofected with vector expressing SpCas9, gRNAs (pEx47A-g1 and g2), and GFP show genome cleavage at DMD pseudo-exon 47A. Red arrowheads point to cleavage products. M, marker; bp, base pair. (FIG. 7B) DNA sequences of DMD pseudo-exon 47A from GPF+ DMD Del iPSCs edited by SpCas9 and the guide RNA pEx47A-g1 and g2. PCR products from genomic DNA of a mixture of myoedited DMD iPSCs were subcloned and sequenced as described above. Uncorrected pseudo-exon 47A sequence is on the top and representative edited sequences are on the bottom. Deleted sequences are replaced by black dashes. Red lower case letter (g) indicate point mutation in the cryptic splice acceptor site. The number of deleted nucleotides is indicated by (-). FIG. 7B discloses SEQ ID NOS 2563-2567, respectively, in order of appearance. (FIG. 7C) Sequence of the lower RT-PCR bands from Fig. 2G (pEx and pEx-Cor. lanes) confirms skipping of pseudo-exon 47A, which reframed the DMD ORF (dystrophin transcript from exons 47 to 48). FIG. 7C discloses SEQ ID NOS 2568-2569, respectively, in order of appearance. (FIG. 7D) Immunocytochemistry shows dystrophin expression in iPSC-derived cardiomyocyte mixtures (pEx-Cor.) and single colony (pEx-Cor-SC) following SpCas9-mediated exon skipping with guide RNA pEx47A-g2 compared to WT and uncorrected cardiomyocyte (pEx). Green, dystrophin staining; red, troponin I staining; blue, nuclei staining. Scale bar = 100 μm.

FIG. 8A-8E. Correction of a large duplication mutation (Dup. Ex55-59) in DMD iPSCs and iPSC-derived cardiomyocytes. (FIG. 8A) This insertion site (In59-In54 junction) was confirmed by PCR using a forward primer targeting intron 59 (F2) and a reverse primer targeting intron 54 (F1) (Fig. 2H and Table 4). The duplication-specific PCR band was absent in WT cells and was presented in Dup cells. (FIG. 8B) T7E1 assays using 293 cells with vector expressing SpCas9, gRNAs (In54-g1, g2 and g3), and GFP show genome cleavage at DMD intron 54. Red arrowheads point to cleavage products. M, marker; bp, base pair. (FIG. 8C) mRNA with duplicated exons was semi-quantified by RT-PCR using the primers flanking the duplication borders exon 53 and exon 55 (Ex53F, a forward primer in exon 53 and Ex59R, a reverse primer in exon 59). Similarly, duplicated exons was semi-quantified by RT-PCR using the primers flanking the duplication borders exon 59 and exon 60 (Ex59F, a forward primer in exon 59 and Ex60R, a reverse primer in exon 60). The duplication-specific RT-PCR upper

bands (red arrowhead) were absent in WT cells and were decreased dramatically in Dup-Cor. cells. (FIG. 8D) PCR results of three representative corrected single colonies (Dup-Cor-SC #4, 6 and 26) and the uncorrected control (Dup). The absence of a duplication-specific PCR band (F2-R1) in colonies 4, 6 and 26 confirmed the deletion of the duplicated DNA region. M denotes size marker lane. bp indicates the length of the marker bands. (FIG. 8E) Immunocytochemistry shows dystrophin expression in iPSC-derived cardiomyocyte mixtures (Dup-Cor.) and single colony (Dup-Cor-SC #6) following SpCas9-mediated exon skipping with guide RNA In54-g1 compared to WT and uncorrected cardiomyocyte (Dup). Green, dystrophin staining; red, troponin I staining; blue, nuclei staining. Scale bar = 100 μm.

DETAILED DESCRIPTION

DMD is a new mutation syndrome with more than 4,000 independent mutations that have been identified in humans (world-wide web at dmd.nl). The majority of patient mutations include deletions that cluster in a hotspot, and thus a therapeutic approach for skipping certain exon applies to large group of patients. The rationale of the exon skipping approach is based on the genetic difference between DMD and Becker muscular dystrophy (BMD) patients. In DMD patients, the reading frame of dystrophin mRNA is disrupted resulting in prematurely truncated, non-functional dystrophin proteins. BMD patients have mutations in the DMD gene that maintain the reading frame allowing the production of internally deleted, but partially functional dystrophins leading to much milder disease symptoms compared to DMD patients.

Duchenne muscular dystrophy (DMD) afflicts ~1 in 5000 males and is caused by mutations in the X-linked dystrophin gene (DMD). These mutations include large deletions, large duplications, point mutations, and other small mutations. The rod-shaped dystrophin protein links the cytoskeleton and the extracellular matrix of muscle cells and maintains the integrity of the plasma membrane. In its absence, muscle cells degenerate. Although DMD causes many severe symptoms, dilated cardiomyopathy is a leading cause of death of DMD patients.

CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9)–mediated genome editing is emerging as a promising tool for correction of genetic disorders. Briefly, an engineered RNA-guided nuclease, such as Cas9 or Cpf1, generates a double-strand break (DSB) at the targeted genomic locus adjacent to a short protospacer adjacent motif (PAM) sequence. There are three primary pathways to repair the DSB: (i) Nonhomologous end joining (NHEJ) directly ligates two DNA ends and leads to

imprecise insertion/deletion (indel) mutations. (ii) Homology-directed repair (HDR) uses sister chromatid or exogenous DNA as a repair template and generates a precise modification at the target sites. (iii) Microhomology-mediated end joining (MMEJ) uses short sequences of nucleotide homology (5 to 25 base pairs) flanking the original DSB to ligate the broken ends and deletes the region between the microhomologies. Although NHEJ can effectively generate indel mutations in most cell types, HDR- or MMEJ-mediated editing is generally thought to be restricted to proliferating cells.

Internal in-frame deletions of dystrophin are associated with Becker muscular dystrophy (BMD), a relatively mild form of muscular dystrophy. Inspired by the attenuated clinical severity of BMD versus DMD, exon skipping has been advanced as a therapeutic strategy to bypass mutations that disrupt the dystrophin open reading frame by modulating splicing patterns of the DMD gene. Several recent studies used CRISPR/Cas9-mediated genome editing to correct various types of DMD mutations in human cells and mice. Some have deployed pairs of guide RNAs to correct the mutation, which requires simultaneous cutting of DNA and excision of large intervening genomic sequences (23 to 725 kb). Fortuitously, the PAM sequence for Streptococcus pyogenes Cas9 (SpCas9), the first and most widely used form of Cas9, contains NAG or NGG, corresponding to the universal splice acceptor sequence (AG) and most of the donor sequences (GG). Thus, in principle, directing Cas9 to splice junctions and the elimination of these consensus sequences by indels can allow for efficient exon skipping. In addition, only a single cleavage of DNA, which disrupts the splice site, can enable skipping of an entire exon.

Given the thousands of individual DMD mutations that have been identified in humans, an obvious question is how such a large number of mutations might be corrected by CRISPR/Cas9-mediated genome editing. Human DMD mutations are clustered in specific "hotspot" areas of the gene (exons 45 to 55 and exons 2 to 10) such that skipping 1 or 2 of 12 targeted exons within or nearby the hotspots (termed "top 12 exons") can, in principle, rescue dystrophin function in a majority (~60%) of DMD patients. Here, CRISPR/Cas9 is used with single-guide RNAs to destroy the conserved splice acceptor or donor sites preceding DMD mutations or to bypass mutant or out-of-frame exons, thereby allowing splicing between surrounding exons to recreate in-frame dystrophin proteins lacking the mutations. This approach was first tested by screening for optimal guide RNAs capable of inducing skipping of the DMD 12 exons that would potentially allow skipping of the most commonly mutated or out-of-frame exons within nearby mutational hotspots. As examples of this approach, the

restoration of dystrophin expression is demonstrated in induced pluripotent stem cell (iPSC)-derived cardiomyocytes harboring exon deletions and a pseudo-exon point mutation. Finally, human iPSC-derived three-dimensional (3D) engineered heart muscle (EHM) was used to test the efficacy of gene editing to overcome abnormalities in cardiac contractility associated with DMD. Contractile dysfunction was observed in DMD EHM, recapitulating the dilated cardiomyopathy (DCM) clinical phenotype of DMD patients, and contractile function was effectively restored in corrected DMD EHM. Thus, genome editing represents a powerful means of eliminating the genetic cause and correcting the muscle and cardiac abnormalities associated with DMD.

These and other aspects of the disclosure are described in further detail below.

CRISPR Systems

CRISPRs (clustered regularly interspaced short palindromic repeats) are DNA loci containing short repetitions of base sequences. Each repetition is followed by short segments of "spacer DNA" from previous exposures to a virus. CRISPRs are found in approximately 40% of sequenced eubacteria genomes and 90% of sequenced archaea. CRISPRs are often associated with Cas genes that code for proteins related to CRISPRs. The CRISPR/Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. CRISPR spacers recognize and silence these exogenous genetic elements like RNAi in eukaryotic organisms.

CRISPR repeats range in size from 24 to 48 base pairs. They usually show some dyad symmetry, implying the formation of a secondary structure such as a hairpin, but are not truly palindromic. Repeats are separated by spacers of similar length. Some CRISPR spacer sequences exactly match sequences from plasmids and phages, although some spacers match the prokaryote's genome (self-targeting spacers). New spacers can be added rapidly in response to phage infection.

Guide RNA (gRNA). As an RNA guided protein, Cas9 requires a short RNA to direct the recognition of DNA targets. Though Cas9 preferentially interrogates DNA sequences containing a PAM sequence NGG it can bind here without a protospacer target. However, the Cas9-gRNA complex requires a close match to the gRNA to create a double strand break. CRISPR sequences in bacteria are expressed in multiple RNAs and then processed to create guide strands for RNA. Because Eukaryotic systems lack some of the proteins required to process CRISPR RNAs the synthetic construct gRNA was created to combine the essential

pieces of RNA for Cas9 targeting into a single RNA expressed with the RNA polymerase type III promoter U6. Synthetic gRNAs are slightly over 100 bp at the minimum length and contain a portion which is targets the 20 protospacer nucleotides immediately preceding the PAM sequence NGG; gRNAs do not contain a PAM sequence.

In some embodiments, the gRNA targets a site within a wildtype dystrophin gene. An exemplary wildtype dystrophin gene includes the human sequence (see GenBank Accession NO. NC_000023.11), located on the human X chromosome, which codes for the protein dystrophin (GenBank Accession No. AAA53189; SEQ ID NO: 5), the sequence of which is reproduced below:

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   1 MLWWEEVEDC YEREDVQKKT FTKWVNAQFS KFGKQHIENL FSDLQDGRRL LDLLEGLTGQ
  61 KLPKEKGSTR VHALNNVNKA LRVLQNNNVD LVNIGSTDIV DGNHKLTLGL IWNIILHWQV
 121 KNVMKNIMAG LQQTNSEKIL LSWVRQSTRN YPQVNVINFT TSWSDGLALN ALIHSHRPDL
 181 FDWNSVVCQQ SATQRLEHAF NIARYQLGIE KLLDPEDVDT TYPDKKSILM YITSLFQVLP
 241 QQVSIEAIQE VEMLPRPPKV TKEEHFQLHH QMHYSQQITV SLAQGYERTS SPKPRFKSYA
 301 YTQAAYVTTS DPTRSPFPSQ HLEAPEDKSF GSSLMESEVN LDRYQTALEE VLSWLLSAED
 361 TLQAQGEISN DVEVVKDQFH THEGYMMDLT AHQGRVGNIL QLGSKLIGTG KLSEDEETEV
 421 QEQMNLLNSR WECLRVASME KQSNLHRVLM DLQNQKLKEL NDWLTKTEER TRKMEEEPLG
 481 PDLEDLKRQV QQHKVLQEDL EQEQVRVNSL THMVVVVDES SGDHATAALE EQLKVLGDRW
 541 ANICRWTEDR WVLLQDILLK WQRLTEEQCL FSAWLSEKED AVNKIHTTGF KDQNEMLSSL
 601 QKLAVLKADL EKKKQSMGKL YSLKQDLLST LKNKSVTQKT EAWLDNFARC WDNLVQKLEK
 661 STAQISQAVT TTQPSLTQTT VMETVTTVTT REQILVKHAQ EELPPPPPQK KRQITVDSEI
 721 RKRLDVDITE LHSWITRSEA VLQSPEFAIF RKEGNFSDLK EKVNAIEREK AEKFRKLQDA
 781 SRSAQALVEQ MVNEGVNADS IKQASEQLNS RWIEFCQLLS ERLNWLEYQN NIIAFYNQLQ
 841 QLEQMTTTAE NWLKIQPTTP SEPTAIKSQL KICKDEVNRL SGLQPQIERL KIQSIALKEK
 901 GQGPMFLDAD FVAFTNHFKQ VFSDVQAREK ELQTIFDTLP PMRYQETMSA IRTWVQQSET
 961 KLSIPQLSVT DYEIMEQRLG ELQALQSSLQ EQQSGLYYLS TTVKEMSKKA PSEISRKYQS
1021 EFEEIEGRWK KLSSQLVEHC QKLEEQMNKL RKIQNHIQTL KKWMAEVDVF LKEEWPALGD
1081 SEILKKQLKQ CRLLVSDIQT IQPSLNSVNE GGQKIKNEAE PEFASRLETE LKELNTQWDH
1141 MCQQVYARKE ALKGGLEKTV SLQKDLSEMH EWMTQAEEEY LERDFEYKTP DELQKAVEEM
1201 KRAKEEAQQK EAKVKLLTES VNSVIAQAPP VAQEALKKEL ETLTTNYQWL CTRLNGKCKT
1261 LEEVWACWHE LLSYLEKANK WLNEVEFKLK TTENIPGGAE EISEVLDSLE NLMRHSEDNP
1321 NQIRILAQTL TDGGVMDELI NEELETFNSR WRELHEEAVR RQKLLEQSIQ SAQETEKSLH
1381 LIQESLTFID KQLAAYIADK VDAAQMPQEA QKIQSDLTSH EISLEEMKKH NQGKEAAQRV
1441 LSQIDVAQKK LQDVSMKFRL FQKPANFELR LQESKMILDE VKMHLPALET KSVEQEVVQS
1501 QLNHCVNLYK SLSEVKSEVE MVIKTGRQIV QKKQTENPKE LDERVTALKL HYNELGAKVT
1561 ERKQQLEKCL KLSRKMRKEM NVLTEWLAAT DMELTKRSAV EGMPSNLDSE VAWGKATQKE
1621 IEKQKVHLKS ITEVGEALKT VLGKKETLVE DKLSLLNSNW IAVTSRAEEW LNLLLEYQKH
1681 METFDQNVDH ITKWIIQADT LLDESEKKKP QQKEDVLKRL KAELNDIRPK VDSTRDQAAN
1741 LMANRGDHCR KLVEPQISEL NHRFAAISHR IKTGKASIPL KELEQFNSDI QKLLEPLEAE
1801 IQQGVNLKEE DFNKDMNEDN EGTVKELLQR GDNLQQRITD ERKREEIKIK QQLLQTKHNA
1861 LKDLRSQRRK KALEISHQWY QYKRQADDLL KCLDDIEKKL ASLPEPRDER KIKEIDRELQ
1921 KKKEELNAVR RQAEGLSEDG AAMAVEPTQI QLSKRWREIE SKFAQFRRLN FAQIHTVREE
1981 TMMVMTEDMP LEISYVPSTY LTEITHVSQA LLEVEQLLNA PDLCAKDFED LFKQEESLKN
2041 IKDSLQQSSG RIDIIHSKKT AALQSATPVE RVKLQEALSQ LDFQWEKVNK MYKDRQGRFD
2101 RSVEKWRRFH YDIKIFNQWL TEAEQFLRKT QIPENWEHAK YKWYLKELQD GIGQRQTVVR
2161 TLNATGEEII QQSSKTDASI LQEKLGSLNL RWQEVCKQLS DRKKRLEEQK NILSEFQRDL
2221 NEFVLWLEEA DNIASIPLEP GKEQQLKEKL EQVKLLVEEL PLRQGILKQL NETGGPVLVS
2281 APISPEEQDK LENKLKQTNL QWIKVSRALP EKQGEIEAQI KDLGQLEKKL EDLEEQLNHL
2341 LLWLSPIRNQ LEIYNQPNQE GPFDVQETEI AVQAKQPDVE EILSKGQHLY KEKPATQPVK
2401 RKLEDLSSEW KAVNRLLQEL RAKQPDLAPG LTTIGASPTQ TVTLVTQPVV TKETAISKLE
2461 MPSSLMLEVP ALADFNRAWT ELTDWLSLLD QVIKSQRVMV GDLEDINEMI IKQKATMQDL
2521 EQRRPQLEEL ITAAQNLKNK TSNQEARTII TDRIERIQNQ WDEVQEHLQN RRQQLNEMLK
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2581 DSTQWLEAKE EAEQVLGQAR AKLESWKEGP YTVDAIQKKI TETKQLAKDL RQWQTNVDVA
2641 NDLALKLLRD YSADDTRKVH MITENINASW RSIHKRVSER EAALEETHRL LQQFPLDLEK
2701 FLAWLTEAET TANVLQDATR KERLLEDSKG VKELMKQWQD LQGEIEAHTD VYHNLDENSQ
2761 KILRSLEGSD DAVLLQRRLD NMNFKWSELR KKSLNIRSHL EASSDQWKRL HLSLQELLVW
2821 LQLKDDELSR QAPIGGDFPA VQKQNDVHRA FKRELKTKEP VIMSTLETVR IFLTEQPLEG
2881 LEKLYQEPRE LPPEERAQNV TRLLRKQAEE VNTEWEKLNL HSADWQRKID ETLERLQELQ
2941 EATDELDLKL RQAEVIKGSW QPVGDLLIDS LQDHLEKVKA LRGEIAPLKE NVSHVNDLAR
3001 QLTTLGIQLS PYNLSTLEDL NTRWKLLQVA VEDRVRQLHE AHRDFGPASQ HFLSTSVQGP
3061 WERAISPNKV PYYINHETQT TCWDHPKMTE LYQSLADLNN VRFSAYRTAM KLRRLQKALC
3121 LDLLSLSAAC DALDQHNLKQ NDQPMDILQI INCLTTIYDR LEQEHNNLVN VPLCVDMCLN
3181 WLLNVYDTGR TGRIRVLSFK TGIISLCKAH LEDKYRYLFK QVASSTGFCD QRRLGLLLHD
3241 SIQIPRQLGE VASFGGSNIE PSVRSCFQFA NNKPEIEAAL FLDWMRLEPQ SMVWLPVLHR
3301 VAAAETAKHQ AKCNICKECP IIGFRYRSLK HFNYDICQSC FFSGRVAKGH KMHYPMVEYC
3361 TPTTSGEDVR DFAKVLKNKF RTKRYFAKHP RMGYLPVQTV LEGDNMETPV TLINFWPVDS
3421 APASSPQLSH DDTHSRIEHY ASRLAEMENS NGSYLNDSIS PNESIDDEHL LIQHYCQSLN
3481 QDSPLSQPRS PAQILISLES EERGELERIL ADLEEENRNL QAEYDRLKQQ HEHKGLSPLP
3541 SPPEMMPTSP QSPRDAELIA EAKLLRQHKG RLEARMQILE DHNKQLESQL HRLRQLLEQP
3601 QAEAKVNGTT VSSPSTSLQR SDSSQPMLLR VVGSQTSDSM GEEDLLSPPQ DTSTGLEEVM
3661 EQLNNSFPSS RGRNTPGKPM REDTM.

In some embodiments, the gRNA targets a site within a mutant dystrophin gene. In some embodiments, the gRNA targets a dystrophin intron. In some embodiments, the gRNA targets a dystrophin exon. In some embodiments, the gRNA targets a site in a dystrophin exon that is expressed and is present in one or more of the dystrophin isoforms shown in Table 1. In embodiments, the gRNA targets a dystrophin splice site. In some embodiments, the gRNA targets a splice donor site on the dystrophin gene. In embodiments, the gRNA targets a splice acceptor site on the dystrophin gene.

Table 1: Dystrophin isoforms

Sequence Name	Nucleic Acid Accession No.	Nucleic Acid SEQ ID NO:	Protein Accession No.	Protein SEQ ID NO:	Description
DMD Genomic Sequence	NC_000023.11 (positions 31119219 to 33339609)	None	None	None	Sequence from Human X Chromosome (at positions Xp21.2 to p21.1) from Assembly GRCh38.p7 (GCF_000001405.33)
Dystrophin Dp427c isoform	NM_000109.3	6	NP_000100.2	7	Transcript Variant: transcript Dp427c is expressed predominantly in neurons of the cortex and the CA regions of the hippocampus. It uses a unique promoter/exon 1 located about 130 kb upstream of the Dp427m transcript promoter. The transcript includes the common exon 2 of transcript Dp427m and has a similar length of 14 kb. The Dp427c isoform contains a unique N-terminal MED sequence, instead of the MLWWEEVEDCY sequence (SEQ ID NO:2476) of isoform Dp427m. The remainder of isoform Dp427c is identical to isoform Dp427m.
Dystrophin Dp427m isoform	NM_004006.2	8	NP_003997.1	9	Transcript Variant: transcript Dp427m encodes the main dystrophin protein found in muscle. As a result of alternative promoter use, exon 1 encodes a unique N-terminal MLWWEEVEDCY (SEQ ID NO: 2476) aa sequence.
Dystrophin Dp427p1 isoform	NM_004009.3	10	NP_004000.1	11	Transcript Variant: transcript Dp427p1 initiates from a unique promoter/exon 1 located in what corresponds to the first intron of transcript Dp427m. The transcript adds the common exon 2 of Dp427m and has a similar length (14 kb). The Dp427p1 isoform replaces the MLWWEEVEDCY

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					(SEQ ID NO: 2476) -start of Dp427m with a unique N-terminal MSEVSSD (SEQ ID NO: 2477) aa sequence.
Dystrophin Dp260-1 isoform	NM_004011.3	12	NP_004002.2	13	Transcript Variant: transcript Dp260-1 uses exons 30-79, and originates from a promoter/exon 1 sequence located in intron 29 of the dystrophin gene. As a result, Dp260-1 contains a 95 bp exon 1 encoding a unique N-terminal 16 aa MTEIILLIFFPAYFLN-sequence (SEQ ID NO: 2478) that replaces amino acids 1-1357 of the full-length dystrophin product (Dp427m isoform).
Dystrophin Dp260-2 isoform	NM_004012.3	14	NP_004003.1	15	Transcript Variant: transcript Dp260-2 uses exons 30-79, starting from a promoter/exon 1 sequence located in intron 29 of the dystrophin gene that is alternatively spliced and lacks N-terminal amino acids 1-1357 of the full length dystrophin (Dp427m isoform). The Dp260-2 transcript encodes a unique N-terminal MSARKLRNLSYKK sequence (SEQ ID NO: 2479).
Dystrophin Dp140 isoform	NM_004013.2	16	NP_004004.1	17	Transcript Variant: Dp140 transcripts use exons 45-79, starting at a promoter/exon 1 located in intron 44. Dp140 transcripts have a long (1 kb) 5' UTR since translation is initiated in exon 51 (corresponding to aa 2461 of dystrophin). In addition to the alternative promoter and exon 1, differential splicing of exons 71-74 and 78 produces at least five Dp140 isoforms. Of these, this transcript (Dp140) contains all of the exons.
Dystrophin Dp116 isoform	NM_004014.2	18	NP_004005.1	19	Transcript Variant: transcript Dp116 uses exons 56-79, starting from a promoter/exon 1 within intron 55. As a result, the Dp116 isoform contains a unique N-terminal MLHRKTYHVK aa sequence (SEQ ID NO: 2480), instead of aa 1-2739 of

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					dystrophin. Differential splicing produces several Dp116-subtypes. The Dp116 isoform is also known as S-dystrophin or apo-dystrophin-2.
Dystrophin Dp71 isoform	NM_004015.2	20	NP_004006.1	21	Transcript Variant: Dp71 transcripts use exons 63-79 with a novel 80- to 100-nt exon containing an ATG start site for a new coding sequence of 17 nt. The short coding sequence is in-frame with the consecutive dystrophin sequence from exon 63. Differential splicing of exons 71 and 78 produces at least four Dp71 isoforms. Of these, this transcript (Dp71) includes both exons 71 and 78.
Dystrophin Dp71b isoform	NM_004016.2	22	NP_004007.1	23	Transcript Variant: Dp71 transcripts use exons 63-79 with a novel 80- to 100-nt exon containing an ATG start site for a new coding sequence of 17 nt. The short coding sequence is in-frame with the consecutive dystrophin sequence from exon 63. Differential splicing of exons 71 and 78 produces at least four Dp71 isoforms. Of these, this transcript (Dp71b) lacks exon 78 and encodes a protein with a different C-terminus than Dp71 and Dp71a isoforms.
Dystrophin Dp71a isoform	NM_004017.2	24	NP_004008.1	25	Transcript Variant: Dp71 transcripts use exons 63-79 with a novel 80- to 100-nt exon containing an ATG start site for a new coding sequence of 17 nt. The short coding sequence is in-frame with the consecutive dystrophin sequence from exon 63. Differential splicing of exons 71 and 78 produces at least four Dp71 isoforms. Of these, this transcript (Dp71a) lacks exon 71.
Dystrophin Dp71ab isoform	NM_004018.2	26	NP_004009.1	27	Transcript Variant: Dp71 transcripts use exons 63-79 with a novel 80- to 100-nt exon containing an ATG start site for a new coding sequence of 17 nt. The short coding sequence is in-frame with the

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					consecutive dystrophin sequence from exon 63. Differential splicing of exons 71 and 78 produces at least four Dp71 isoforms. Of these, this transcript (Dp71ab) lacks both exons 71 and 78 and encodes a protein with a C-terminus like isoform Dp71b.
Dystrophin Dp40 isoform	NM_004019.2	28	NP_004010.1	29	Transcript Variant: transcript Dp40 uses exons 63-70. The 5' UTR and encoded first 7 aa are identical to that in transcript Dp71, but the stop codon lies at the splice junction of the exon/intron 70. The 3' UTR includes nt from intron 70 which includes an alternative polyadenylation site. The Dp40 isoform lacks the normal C-terminal end of full-length dystrophin (aa 3409-3685).
Dystrophin Dp140c isoform	NM_004020.3	30	NP_004011.2	31	Transcript Variant: Dp140 transcripts use exons 45-79, starting at a promoter/exon 1 located in intron 44. Dp140 transcripts have a long (1 kb) 5' UTR since translation is initiated in exon 51 (corresponding to aa 2461 of dystrophin). In addition to the alternative promoter and exon 1, differential splicing of exons 71-74 and 78 produces at least five Dp140 isoforms. Of these, this transcript (Dp140c) lacks exons 71-74.
Dystrophin Dp140b isoform	NM_004021.2	32	NP_004012.1	33	Transcript Variant: Dp140 transcripts use exons 45-79, starting at a promoter/exon 1 located in intron 44. Dp140 transcripts have a long (1 kb) 5' UTR since translation is initiated in exon 51 (corresponding to aa 2461 of dystrophin). In addition to the alternative promoter and exon 1, differential splicing of exons 71-74 and 78 produces at least five Dp140 isoforms. Of these, this transcript (Dp140b) lacks exon 78 and encodes a protein with a unique C-terminus.

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Dystrophin Dp140ab isoform	NM_004022.2	34	NP_004013.1	35	Transcript Variant: Dp140 transcripts use exons 45-79, starting at a promoter/exon 1 located in intron 44. Dp140 transcripts have a long (1 kb) 5' UTR since translation is initiated in exon 51 (corresponding to aa 2461 of dystrophin). In addition to the alternative promoter and exon 1, differential splicing of exons 71-74 and 78 produces at least five Dp140 isoforms. Of these, this transcript (Dp140ab) lacks exons 71 and 78 and encodes a protein with a unique C-terminus.
Dystrophin Dp140bc isoform	NM_004023.2	36	NP_004014.1	37	Transcript Variant: Dp140 transcripts use exons 45-79, starting at a promoter/exon 1 located in intron 44. Dp140 transcripts have a long (1 kb) 5' UTR since translation is initiated in exon 51 (corresponding to aa 2461 of dystrophin). In addition to the alternative promoter and exon 1, differential splicing of exons 71-74 and 78 produces at least five Dp140 isoforms. Of these, this transcript (Dp140bc) lacks exons 71-74 and 78 and encodes a protein with a unique C-terminus.
Dystrophin isoform X2	XM_006724469.3	38	XP_006724532.1	39	
Dystrophin isoform X5	XM_011545467.1	40	XP_011543769.1	41	
Dystrophin isoform X6	XM_006724473.2	42	XP_006724536.1	43	
Dystrophin isoform X8	XM_006724475.2	44	XP_006724538.1	45	
Dystrophin isoform X4	XM_017029328.1	46	XP_016884817.1	47	
Dystrophin isoform X1	XM_006724468.2	48	XP_006724531.1	49	

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Dystrophin isoform X13	XM_017029331.1	50	XP_016884820.1	51	
Dystrophin isoform X3	XM_006724470.3	52	XP_006724533.1	53	
Dystrophin isoform X7	XM_006724474.3	54	XP_006724537.1	55	
Dystrophin isoform X9	XM_011545468.2	56	XP_011543770.1	57	
Dystrophin isoform X11	XM_017029330.1	58	XP_016884819.1	59	
Dystrophin isoform X10	XM_017029329.1	865	XP_016884818.1	866	
Dystrophin isoform X12	XM_011545469.1	867	XP_011543771.1	868	

In embodiments, the guide RNA targets a mutant DMD exon. In some embodiments, the mutant exon is exon 23 or 51. In some embodiments, the guide RNA targets at least one of exons 1, 23, 41, 44, 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55 of the dystrophin gene. In embodiments, the guide RNA targets at least one of introns 44, 45, 50, 51, 52, 53, 54, or 55 of the dystrophin gene. In preferred embodiments, the guide RNAs are designed to induce skipping of exon 51 or exon 23. In embodiments, the gRNA is targeted to a splice acceptor site of exon 51 or exon 23.

Suitable gRNAs and genomic target sequences for use in various compositions and methods disclosed herein are provided as SEQ ID NOs: 60-705, 712-862, and 947-2377.

In some embodiments, the gRNA or gRNA target site has a sequence of any one of the gRNAs or gRNA target sites shown in Tables 5-19.

In some embodiments, gRNAs of the disclosure comprise a sequence that is complementary to a target sequence within a coding sequence or a non-coding sequence corresponding to the *DMD* gene, and, therefore, hybridize to the target sequence. In some embodiments, gRNAs for Cpf1 comprise a single crRNA containing a direct repeat scaffold sequence followed by 24 nucleotides of guide sequence. In some embodiments, a "guide" sequence of the crRNA comprises a sequence of the gRNA that is complementary to a target sequence. In some embodiments, crRNA of the disclosure comprises a sequence of the gRNA that is not complementary to a target sequence. "Scaffold" sequences of the disclosure link the gRNA to the Cpf1 polypeptide. "Scaffold" sequences of the disclosure are not equivalent to a tracrRNA sequence of a gRNA-Cas9 construct.

In some embodiments, a nucleic acid may comprise one or more sequences encoding a gRNA. In some embodiments, a nucleic acid may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 sequences encoding a gRNA. In some embodiments, all of the sequences encode the same gRNA. In some embodiments, all of the sequences encode different gRNAs. In some embodiments, at least 2 of the sequences encode the same gRNA, for example at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 of the sequences encode the same gRNA.

Nucleases

Cas Nucleases. CRISPR-associated (*cas*) genes are often associated with CRISPR repeat-spacer arrays. As of 2013, more than forty different Cas protein families had been described. Of these protein families, Cas1 appears to be ubiquitous among different CRISPR/Cas systems. Particular combinations of *cas* genes and repeat structures have been used to define 8 CRISPR subtypes (Ecoli, Ypest, Nmeni, Dvulg, Tneap, Hmari, Apern, and Mtube), some of which are associated with an additional gene module encoding repeat-associated mysterious proteins (RAMPs). More than one CRISPR subtype may occur in a single genome. The sporadic distribution of the CRISPR/Cas subtypes suggests that the system is subject to horizontal gene transfer during microbial evolution.

Exogenous DNA is apparently processed by proteins encoded by Cas genes into small elements (~30 base pairs in length), which are then somehow inserted into the CRISPR locus near the leader sequence. RNAs from the CRISPR loci are constitutively expressed and are processed by Cas proteins to small RNAs composed of individual, exogenously-derived sequence elements with a flanking repeat sequence. The RNAs guide other Cas proteins to silence exogenous genetic elements at the RNA or DNA level. Evidence suggests functional diversity among CRISPR subtypes. The Cse (Cas subtype *Ecoli*) proteins (called CasA-E in *E. coli*) form a functional complex, Cascade, that processes CRISPR RNA transcripts into spacer-repeat units that Cascade retains. In other prokaryotes, Cas6 processes the CRISPR transcripts. Interestingly, CRISPR-based phage inactivation in *E. coli* requires Cascade and Cas3, but not Cas1 and Cas2. The Cmr (Cas RAMP module) proteins found in *Pyrococcus furiosus* and other prokaryotes form a functional complex with small CRISPR RNAs that recognizes and cleaves complementary target RNAs. RNA-guided CRISPR enzymes are classified as type V restriction enzymes.

Cas9 is a nuclease, an enzyme specialized for cutting DNA, with two active cutting sites, one for each strand of the double helix. One or both sites may be deactivated while preserving Cas9's ability to locate its target DNA. Jinek *et al.* (2012) combined tracrRNA and spacer RNA into a "single-guide RNA" molecule that, mixed with Cas9, can find and cut the correct DNA targets and such synthetic guide RNAs are used for gene editing.

Cas9 proteins are highly enriched in pathogenic and commensal bacteria. CRISPR/Cas-mediated gene regulation may contribute to the regulation of endogenous bacterial genes, particularly during bacterial interaction with eukaryotic hosts. For example, Cas protein Cas9

of *Francisella novicida* uses a unique, small, CRISPR/Cas-associated RNA (scaRNA) to repress an endogenous transcript encoding a bacterial lipoprotein that is critical for *F. novicida* to dampen host response and promote virulence. It has been shown that coinjection of Cas9 mRNA and sgRNAs into the germline (zygotes) can be used to generate mice with mutations. Delivery of Cas9 DNA sequences also is contemplated.

The CRISPR/Cas systems are separated into three classes. Class 1 uses several Cas proteins together with the CRISPR RNAs (crRNA) to build a functional endonuclease. Class 2 CRISPR systems use a single Cas protein with a crRNA. Cpf1 has been recently identified as a Class II, Type V CRISPR/Cas system containing a ~1,300 amino acid protein. See also U.S. Patent Publication 2014/0068797, which is incorporated by reference in its entirety.

In some embodiments, the compositions of the disclosure include a small version of a Cas9 from the bacterium *Staphylococcus aureus* (UniProt Accession No. J7RUA5). The small version of the Cas9 provides advantages over wildtype or full length Cas9. In some embodiments the Cas9 is a *Streptococcus pyogenes* (spCas9).

Cpf1 Nucleases. Clustered Regularly Interspaced Short Palindromic Repeats from *Prevotella* and *Francisella* 1 or CRISPR/Cpf1 is a DNA-editing technology which shares some similarities with the CRISPR/Cas9 system. Cpf1 is an RNA-guided endonuclease of a class II CRISPR/Cas system. This acquired immune mechanism is found in *Prevotella* and *Francisella* bacteria. It prevents genetic damage from viruses. Cpf1 genes are associated with the CRISPR locus, coding for an endonuclease that use a guide RNA to find and cleave viral DNA. Cpf1 is a smaller and simpler endonuclease than Cas9, overcoming some of the CRISPR/Cas9 system limitations.

Cpf1 appears in many bacterial species. The ultimate Cpf1 endonuclease that was developed into a tool for genome editing was taken from one of the first 16 species known to harbor it.

In embodiments, the Cpf1 is a Cpf1 enzyme from *Acidaminococcus* (species BV3L6, UniProt Accession No. U2UMQ6; SEQ ID NO: 870), having the sequence set forth below:

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  1 MTQFEGFTNL YQVSKTLRFE LIPQGKTLKH IQEQGFIEED KARNDHYKEL KPIIDRIYKT
 61 YADQCLQLVQ LDWENLSAAI DSYRKEKTEE TRNALIEEQA TYRNAIHDYF IGRTDNLTDA
121 INKRHAEIYK GLFKAELFNG KVLKQLGTVT TTEHENALLR SFDKFTTYFS GFYENRKNVF
181 SAEDISTAIP HRIVQDNFPK FKENCHIFTR LITAVPSLRE HFENVKKAIG IFVSTSIEEV
241 FSFPFYNQLL TQTQIDLYNQ LLGGISREAG TEKIKGLNEV LNLAIQKNDE TAHIIASLPH
301 RFIPLFKQIL SDRNTLSFIL EEFKSDEEVI QSFCKYKTLL RNENVLETAE ALFNELNSID
361 LTHIFISHKK LETISSALCD HWDTLRNALY ERRISELTGK ITKSAKEKVQ RSLKHEDINL
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 421 QEIISAAGKE LSEAFKQKTS EILSHAHAAL DQPLPTTLKK QEEKEILKSQ LDSLLGLYHL
 481 LDWFAVDESN EVDPEFSARL TGIKLEMEPS LSFYNKARNY ATKKPYSVEK FKLNFQMPTL
 541 ASGWDVNKEK NNGAILFVKN GLYYLGIMPK QKGRYKALSF EPTEKTSEGF DKMYYDYFPD
 601 AAKMIPKCST QLKAVTAHFQ THTTPILLSN NFIEPLEITK EIYDLNNPEK EPKKFQTAYA
 661 KKTGDQKGYR EALCKWIDFT RDFLSKYTKT TSIDLSSLRP SSQYKDLGEY YAELNPLLYH
 721 ISFQRIAEKE IMDAVETGKL YLFQIYNKDF AKGHHGKPNL HTLYWTGLFS PENLAKTSIK
 781 LNGQAELFYR PKSRMKRMAH RLGEKMLNKK LKDQKTPIPD TLYQELYDYV NHRLSHDLSD
 841 EARALLPNVI TKEVSHEIIK DRRFTSDKFF FHVPITLNYQ AANSPSKFNQ RVNAYLKEHP
 901 ETPIIGIDRG ERNLIYITVI DSTGKILEQR SLNTIQQFDY QKKLDNREKE RVAARQAWSV
 961 VGTIKDLKQG YLSQVIHEIV DLMIHYQAVV VLENLNFGFK SKRTGIAEKA VYQQFEKMLI
1021 DKLNCLVLKD YPAEKVGGVL NPYQLTDQFT SFAKMGTQSG FLFYVPAPYT SKIDPLTGFV
1081 DPFVWKTIKN HESRKHFLEG FDFLHYDVKT GDFILHFKMN RNLSFQRGLP GFMPAWDIVF
1141 EKNETQFDAK GTPFIAGKRI VPVIENHRFT GRYRDLYPAN ELIALLEEKG IVFRDGSNIL
1201 PKLLENDDSH AIDTMVALIR SVLQMRNSNA ATGEDYINSP VRDLNGVCFD SRFQNPEWPM
1261 DADANGAYHI ALKGQLLLNH LKESKDLKLQ NGISNQDWLA YIQELRN
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In some embodiments, the Cpf1 is a Cpf1 enzyme from *Lachnospiraceae* (species ND2006, UniProt Accession No. A0A182DWE3; SEQ ID NO: 871), having the sequence set forth below:

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   1 AASKLEKFTN CYSLSKTLRF KAIPVGKTQE NIDNKRLLVE DEKRAEDYKG VKKLLDRYYL
  61 SFINDVLHSI KLKNLNNYIS LFRKKTRTEK ENKELENLEI NLRKEIAKAF KGAAGYKSLF
 121 KKDIIETILP EAADDKDEIA LVNSFNGFTT AFTGFFDNRE NMFSEEAKST SIAFRCINEN
 181 LTRYISNMDI FEKVDAIFDK HEVQEIKEKI LNSDYDVEDF FEGEFFNFVL TQEGIDVYNA
 241 IIGGFVTESG EKIKGLNEYI NLYNAKTKQA LPKFKPLYKQ VLSDRESLSF YGEGYTSDEE
 301 VLEVFRNTLN KNSEIFSSIK KLEKLFKNFD EYSSAGIFVK NGPAISTISK DIFGEWNLIR
 361 DKWNAEYDDI HLKKKAVVTE KYEDDRRKSF KKIGSFSLEQ LQEYADADLS VVEKLKEIII
 421 QKVDEIYKVY GSSEKLFDAD FVLEKSLKKN DAVVAIMKDL LDSVKSFENY IKAFFGEGKE
 481 TNRDESFYGD FVLAYDILLK VDHIYDAIRN YVTQKPYSKD KFKLYFQNPQ FMGGWDKDKE
 541 TDYRATILRY GSKYYLAIMD KKYAKCLQKI DKDDVNGNYE KINYKLLPGP NKMLPKVFFS
 601 KKWMAYYNPS EDIQKIYKNG TFKKGDMFNL NDCHKLIDFF KDSISRYPKW SNAYDFNFSE
 661 TEKYKDIAGF YREVEEQGYK VSFESASKKE VDKLVEEGKL YMFQIYNKDF SDKSHGTPNL
 721 HTMYFKLLFD ENNHGQIRLS GGAELFMRRA SLKKEELVVH PANSPIANKN PDNPKKTTTL
 781 SYDVYKDKRF SEDQYELHIP IAINKCPKNI FKINTEVRVL LKHDDNPYVI GIDRGERNLL
 841 YIVVVDGKGN IVEQYSLNEI INNFNGIRIK TDYHSLLDKK EKERFEARQN WTSIENIKEL
 901 KAGYISQVVH KICELVEKYD AVIALEDLNS GFKNSRVKVE KQVYQKFEKM LIDKLNYMVD
 961 KKSNPCATGG ALKGYQITNK FESFKSMSTQ NGFIFYIPAW LTSKIDPSTG FVNLLKTKYT
1021 SIADSKKFIS SFDRIMYVPE EDLFEFALDY KNFSRTDADY IKKWKLYSYG NRIRIFAAAK
1081 KNNVFAWEEV CLTSAYKELF NKYGINYQQG DIRALLCEQS DKAFYSSFMA LMSLMLQMRN
1141 SITGRTDVDF LISPVKNSDG IFYDSRNYEA QENAILPKNA DANGAYNIAR KVLWAIGQFK
1201 KAEDEKLDKV KIAISNKEWL EYAQTSVK
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In some embodiments, the Cpf1 is codon optimized for expression in mammalian cells. In some embodiments, the Cpf1 is codon optimized for expression in human cells or mouse cells.

The Cpf1 locus contains a mixed alpha/beta domain, a RuvC-I followed by a helical region, a RuvC-II and a zinc finger-like domain. The Cpf1 protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9. Furthermore, Cpf1 does not have a HNH endonuclease domain, and the N-terminal of Cpf1 does not have the alpha-helical recognition lobe of Cas9.

Cpf1 CRISPR-Cas domain architecture shows that Cpf1 is functionally unique, being classified as Class 2, type V CRISPR system. The Cpf1 loci encode Cas1, Cas2 and Cas4 proteins more similar to types I and III than from type II systems. Database searches suggest the abundance of Cpf1-family proteins in many bacterial species.

Functional Cpf1 does not require a tracrRNA, therefore, only crRNA is required. This benefits genome editing because Cpf1 is not only smaller than Cas9, but also it has a smaller sgRNA molecule (approximately half as many nucleotides as Cas9).

The Cpf1-crRNA complex cleaves target DNA or RNA by identification of a protospacer adjacent motif 5'-YTN-3' (where "Y" is a pyrimidine and "N" is any nucleobase) or 5'-TTN-3', in contrast to the G-rich PAM targeted by Cas9. After identification of PAM, Cpf1 introduces a sticky-end-like DNA double-stranded break of 4 or 5 nucleotides overhang.

The CRISPR/Cpf1 system consist of a Cpf1 enzyme and a guide RNA that finds and positions the complex at the correct spot on the double helix to cleave target DNA. CRISPR/Cpf1 systems activity has three stages:

Adaptation, during which Cas1 and Cas2 proteins facilitate the adaptation of small fragments of DNA into the CRISPR array;

Formation of crRNAs: processing of pre-cr-RNAs producing of mature crRNAs to guide the Cas protein; and

Interference, in which the Cpf1 is bound to a crRNA to form a binary complex to identify and cleave a target DNA sequence.

Cas9 *versus* Cpf1. Cas9 requires two RNA molecules to cut DNA while Cpf1 needs one. The proteins also cut DNA at different places, offering researchers more options when selecting an editing site. Cas9 cuts both strands in a DNA molecule at the same position, leaving behind ‘blunt’ ends. Cpf1 leaves one strand longer than the other, creating 'sticky' ends that are easier to work with. Cpf1 appears to be more able to insert new sequences at the cut site, compared to Cas9. Although the CRISPR/Cas9 system can efficiently disable genes, it is challenging to insert genes or generate a knock-in. Cpf1 lacks tracrRNA, utilizes a T-rich PAM and cleaves DNA via a staggered DNA DSB.

In summary, important differences between Cpf1 and Cas9 systems are that Cpf1 recognizes different PAMs, enabling new targeting possibilities, creates 4-5 nt long sticky ends,

instead of blunt ends produced by Cas9, enhancing the efficiency of genetic insertions and specificity during NHEJ or HDR, and cuts target DNA further away from PAM, further away from the Cas9 cutting site, enabling new possibilities for cleaving the DNA.

Table 2: Differences between Cas9 and Cpf1

Feature	Cas9	Cpf1
Structure	Two RNA required (Or 1 fusion transcript (crRNA+tracrRNA=gRNA))	One RNA required
Cutting mechanism	Blunt end cuts	Staggered end cuts
Cutting site	Proximal to recognition site	Distal from recognition site
Target sites	G-rich PAM	T-rich PAM

Other Nucleases. In some embodiments, the nuclease is a Cas9 or a Cpf1 nuclease. In addition to Cas9 nucleases and Cpf1 nucleases, other nucleases may be used in the compositions and methods of the disclosure. For example, in some embodiments, the nuclease is a Type II, Type V-A, Type V-B, Type V-C, Type V-U, Type VI-B nuclease. In some embodiments, the nuclease is a Cas9, Cas12a, Cas12b, Cas12c, Tnp-B like, Cas13a (C2c2), or Cas13b nuclease. In some embodiments, the nuclease is a TAL nuclease, a meganuclease, or a zinc-finger nuclease.

CRISPR-mediated gene editing. The first step in editing the DMD gene using CRISPR/Cpf1 or CRISPR/Cas9 (or another nuclease) is to identify the genomic target sequence. The genomic target for the gRNAs of the disclosure can be any approximately 24 nucleotide DNA sequence, provided that the sequence is unique compared to the rest of the genome. In some embodiments, the genomic target sequence corresponds to a sequence within exon 51, exon 45, exon 44, exon 53, exon 46, exon 52, exon 50, exon 43, exon 6, exon 7, exon 8, and/or exon 55 of the human dystrophin gene. In some embodiments, the genomic target sequence is a 5' or 3' splice site of exon 51, exon 45, exon 44, exon 53, exon 46, exon 52, exon 50, exon 43, exon 6, exon 7, exon 8, and/or exon 55 of the human dystrophin gene. In some embodiments, the genomic target sequence corresponds to a sequence within an intron immediately upstream or downstream of exon 51, exon 45, exon 44, exon 53, exon 46, exon

52, exon 50, exon 43, exon 6, exon 7, exon 8, and/or exon 55 of the human dystrophin gene. Exemplary genomic target sequences can be found in Tables 2, 6, 8, 10, 12, 14 and 19.

The next step in editing the DMD gene is to identify all Protospacer Adjacent Motif (PAM) sequences within the genetic region to be targeted. The target sequence must be immediately upstream of a PAM. Once all possible PAM sequences and putative target sites have been identified, the next step is to choose which site is likely to result in the most efficient on-target cleavage. The gRNA targeting sequence needs to match the target sequence, and the gRNA targeting sequence must not match additional sites within the genome. In preferred embodiments, the gRNA targeting sequence has perfect homology to the target with no homology elsewhere in the genome. In some embodiments, a given gRNA targeting sequence will have additional sites throughout the genome where partial homology exists. These sites are called "off-targets" and should be considered when designing a gRNA. In general, off-target sites are not cleaved as efficiently when mismatches occur near the PAM sequence, so gRNAs with no homology or those with mismatches close to the PAM sequence will have the highest specificity. In addition to "off-target activity", factors that maximize cleavage of the desired target sequence ("on-target activity") must be considered. It is known to those of skill in the art that two gRNA targeting sequences, each having 100% homology to the target DNA may not result in equivalent cleavage efficiency. In fact, cleavage efficiency may increase or decrease depending upon the specific nucleotides within the selected target sequence. Close examination of predicted on-target and off-target activity of each potential gRNA targeting sequence is necessary to design the best gRNA. Several gRNA design programs have been developed that are capable of locating potential PAM and target sequences and ranking the associated gRNAs based on their predicted on-target and off-target activity (e.g. CRISPRdirect, available at www.crispr.dbcls.jp).

The next step is to synthesize and clone desired gRNAs. Targeting oligos can be synthesized, annealed, and inserted into plasmids containing the gRNA scaffold using standard restriction-ligation cloning. However, the exact cloning strategy will depend on the gRNA vector that is chosen. The gRNAs for Cpf1 are notably simpler than the gRNAs for Cas9, and only consist of a single crRNA containing direct repeat scaffold sequence followed by approximately 24 nucleotides of guide sequence.

Each gRNA should then be validated in one or more target cell lines. For example, after the Cas9 or Cpf1 and the gRNA are delivered to the cell, the genomic target region may

be amplified using PCR and sequenced according to methods known to those of skill in the art.

In some embodiments, gene editing may be performed in vitro or ex vivo. In some embodiments, cells are contacted in vitro or ex vivo with a Cas9 or a Cpf1 and a gRNA that targets a dystrophin splice site. In some embodiments, the cells are contacted with one or more nucleic acids encoding the Cas9 or Cpf1 and the guide RNA. In some embodiments, the one or more nucleic acids are introduced into the cells using, for example, lipofection or electroporation. Gene editing may also be performed in zygotes. In embodiments, zygotes may be injected with one or more nucleic acids encoding Cas9 or Cpf1 and a gRNA that targets a dystrophin splice site. The zygotes may subsequently be injected into a host.

In embodiments, the Cas9 or Cpf1 is provided on a vector. In embodiments, the vector contains a Cas9 derived from S. *pyogenes* (SpCas9, SEQ ID NO. 872). In embodiments, the vector contains a Cas9 derived from S. *aureus* (SaCas9, SEQ ID NO. 873). In embodiments, the vector contains a Cpf1 sequence derived from a *Lachnospiraceae* bacterium. See, for example, Uniprot Accession No. A0A182DWE3; SEQ ID NO. 871. In embodiments, the vector contains a Cpf1 sequence derived from an *Acidaminococcus* bacterium. See, for example, Uniprot Accession No. U2UMQ6; SEQ ID NO. 870. In some embodiments, the Cas9 or Cpf1 sequence is codon optimized for expression in human cells or mouse cells. In some embodiments, the vector further contains a sequence encoding a fluorescent protein, such as GFP, which allows Cas9 or Cpf1-expressing cells to be sorted using fluorescence activated cell sorting (FACS). In some embodiments, the vector is a viral vector such as an adeno-associated viral vector.

In embodiments, the gRNA is provided on a vector. In some embodiments, the vector is a viral vector such as an adeno-associated viral vector. In embodiments, the Cas9 or Cpf1 and the guide RNA are provided on the same vector. In embodiments, the Cas9 or Cpf1 and the guide RNA are provided on different vectors.

In some embodiments, the cells are additionally contacted with a single-stranded DMD oligonucleotide to effect homology directed repair. In some embodiments, small INDELs restore the protein reading frame of dystrophin ("reframing" strategy). When the reframing strategy is used, the cells may be contacted with a single gRNA. In embodiments, a splice donor or splice acceptor site is disrupted, which results in exon skipping and restoration of the

protein reading frame ("exon skipping" strategy). When the exon skipping strategy is used, the cells may be contacted with two or more gRNAs.

Efficiency of in vitro or ex vivo Cas9 or Cpf1-mediated DNA cleavage may be assessed using techniques known to those of skill in the art, such as the T7 E1 assay. Restoration of DMD expression may be confirmed using techniques known to those of skill in the art, such as RT-PCR, western blotting, and immunocytochemistry.

In some embodiments, in vitro or ex vivo gene editing is performed in a muscle or satellite cell. In some embodiments, gene editing is performed in iPSC or iCM cells. In embodiments, the iPSC cells are differentiated after gene editing. For example, the iPSC cells may be differentiated into a muscle cell or a satellite cell after editing. In embodiments, the iPSC cells are differentiated into cardiac muscle cells, skeletal muscle cells, or smooth muscle cells. In embodiments, the iPSC cells are differentiated into cardiomyocytes. iPSC cells may be induced to differentiate according to methods known to those of skill in the art.

In some embodiments, contacting the cell with the Cas9 or the Cpf1 and the gRNA restores dystrophin expression. In embodiments, cells which have been edited in vitro or ex vivo, or cells derived therefrom, show levels of dystrophin protein that is comparable to wildtype cells. In embodiments, the edited cells, or cells derived therefrom, express dystrophin at a level that is 50%, 60%, 70%, 80%, 90%, 95% or any percentage in between of wildtype dystrophin expression levels. In embodiments, the cells which have been edited in vitro or ex vivo, or cells derived therefrom, have a mitochondrial number that is comparable to that of wildtype cells. In embodiments the edited cells, or cells derived therefrom, have 50%, 60%, 70%, 80%, 90%, 95% or any percentage in between as many mitochondria as wildtype cells. In embodiments, the edited cells, or cells derived therefrom, show an increase in oxygen consumption rate (OCR) compared to non-edited cells at baseline.

Nucleic Acid Expression Vectors. As discussed above, in certain embodiments, expression cassettes are employed to express a transcription factor product, either for subsequent purification and delivery to a cell/subject, or for use directly in a genetic-based delivery approach. Provided herein are expression vectors which contain one or more nucleic acids encoding Cas9 or Cpf1 and at least one DMD guide RNA that targets a dystrophin splice site. In some embodiments, a nucleic acid encoding Cas9 or Cpf1 and a nucleic acid encoding at least one guide RNA are provided on the same vector. In further embodiments, a nucleic acid

encoding Cas9 or Cpf1 and a nucleic acid encoding least one guide RNA are provided on separate vectors.

Expression requires that appropriate signals be provided in the vectors, and include various regulatory elements such as enhancers/promoters from both viral and mammalian sources that drive expression of the genes of interest in cells. Elements designed to optimize messenger RNA stability and translatability in host cells also are defined. The conditions for the use of a number of dominant drug selection markers for establishing permanent, stable cell clones expressing the products are also provided, as is an element that links expression of the drug selection markers to expression of the polypeptide.

Throughout this application, the term "expression cassette" is meant to include any type of genetic construct containing a nucleic acid coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed and translated, *i.e.*, is under the control of a promoter. A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. The phrase "under transcriptional control" means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the gene. An "expression vector" is meant to include expression cassettes comprised in a genetic construct that is capable of replication, and thus including one or more of origins of replication, transcription termination signals, poly-A regions, selectable markers, and multipurpose cloning sites.

Regulatory Elements. The term promoter will be used here to refer to a group of transcriptional control modules that are clustered around the initiation site for RNA polymerase II. Much of the thinking about how promoters are organized derives from analyses of several viral promoters, including those for the HSV thymidine kinase (*tk*) and SV40 early transcription units. These studies, augmented by more recent work, have shown that promoters are composed of discrete functional modules, each consisting of approximately 7-20 bp of DNA, and containing one or more recognition sites for transcriptional activator or repressor proteins.

At least one module in each promoter functions to position the start site for RNA synthesis. The best known example of this is the TATA box, but in some promoters lacking a TATA box, such as the promoter for the mammalian terminal deoxynucleotidyl transferase

gene and the promoter for the SV40 late genes, a discrete element overlying the start site itself helps to fix the place of initiation.

RNA Polymerase and Pol III Promoters. In eukaryotes, RNA polymerase III (also called Pol III) transcribes DNA to synthesize ribosomal 5S rRNA, tRNA and other small RNAs. The genes transcribed by RNA Pol III fall in the category of "housekeeping" genes whose expression is required in all cell types and most environmental conditions. Therefore, the regulation of Pol III transcription is primarily tied to the regulation of cell growth and the cell cycle, thus requiring fewer regulatory proteins than RNA polymerase II. Under stress conditions however, the protein Maf1 represses Pol III activity.

In the process of transcription (by any polymerase) there are three main stages: (i) initiation, requiring construction of the RNA polymerase complex on the gene's promoter; (ii) elongation, the synthesis of the RNA transcript; and (iii) termination, the finishing of RNA transcription and disassembly of the RNA polymerase complex.

Promoters under the control of RNA Pol III include those for ribosomal 5S rRNA, tRNA and few other small RNAs such as U6 spliceosomal RNA, RNase P and RNase MRP RNA, 7SL RNA (the RNA component of the signal recognition particles), Vault RNAs, Y RNA, SINEs (short interspersed repetitive elements), 7SK RNA, two microRNAs, several small nucleolar RNAs and several few regulatory antisense RNAs.

Additional Promoters and Elements

In some embodiments, the Cas9 or Cpf1 constructs of the disclosure are expressed by a muscle-cell specific promoter. This muscle-cell specific promoter may be constitutively active or may be an inducible promoter.

Additional promoter elements regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the tk promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either co-operatively or independently to activate transcription.

In certain embodiments, viral promoters such as the human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter, the Rous sarcoma virus long terminal repeat, rat insulin promoter and glyceraldehyde-3-phosphate dehydrogenase can be used to obtain high-level expression of the coding sequence of interest. The use of other viral or mammalian cellular or bacterial phage promoters which are well-known in the art to achieve expression of a coding sequence of interest is contemplated as well, provided that the levels of expression are sufficient for a given purpose. By employing a promoter with well-known properties, the level and pattern of expression of the protein of interest following transfection or transformation can be optimized. Further, selection of a promoter that is regulated in response to specific physiologic signals can permit inducible expression of the gene product.

Enhancers are genetic elements that increase transcription from a promoter located at a distant position on the same molecule of DNA. Enhancers are organized much like promoters. That is, they are composed of many individual elements, each of which binds to one or more transcriptional proteins. The basic distinction between enhancers and promoters is operational. An enhancer region as a whole must be able to stimulate transcription at a distance; this need not be true of a promoter region or its component elements. On the other hand, a promoter must have one or more elements that direct initiation of RNA synthesis at a particular site and in a particular orientation, whereas enhancers lack these specificities. Promoters and enhancers are often overlapping and contiguous, often seeming to have a very similar modular organization.

Below is a list of promoters/enhancers and inducible promoters/enhancers that could be used in combination with the nucleic acid encoding a gene of interest in an expression construct. Additionally, any promoter/enhancer combination (as per the Eukaryotic Promoter Data Base EPDB) could also be used to drive expression of the gene. Eukaryotic cells can support cytoplasmic transcription from certain bacterial promoters if the appropriate bacterial polymerase is provided, either as part of the delivery complex or as an additional genetic expression construct.

The promoter and/or enhancer may be, for example, immunoglobulin light chain, immunoglobulin heavy chain, T-cell receptor, HLA DQ a and/or DQ β, β-interferon, interleukin-2, interleukin-2 receptor, MHC class II 5, MHC class II HLA-Dra, β-Actin, muscle creatine kinase (MCK), prealbumin (transthyretin), elastase I, metallothionein (MTII), collagenase, albumin, α-fetoprotein, t-globin, β-globin, c-fos, c-HA-*ras,* insulin, neural cell

adhesion molecule (NCAM), α_1-antitrypain, H2B (TH2B) histone, mouse and/or type I collagen, glucose-regulated proteins (GRP94 and GRP78), rat growth hormone, human serum amyloid A (SAA), troponin I (TN I), platelet-derived growth factor (PDGF), Duchenne muscular dystrophy, SV40, polyoma, retroviruses, papilloma virus, hepatitis B virus, human immunodeficiency virus, cytomegalovirus (CMV), and gibbon ape leukemia virus.

In some embodiments, inducible elements may be used. In some embodiments, the inducible element is, for example, MTII, MMTV (mouse mammary tumor virus), β-interferon, adenovirus 5 E2, collagenase, stromelysin, SV40, murine MX gene, GRP78 gene, α-2-macroglobulin, vimentin, MHC class I gene H-2κb, HSP70, proliferin, tumor necrosis factor, and/or thyroid stimulating hormone α gene. In some embodiments, the inducer is phorbol ester (TFA), heavy metals, glucocorticoids, poly(rI)x, poly(rc), ElA, phorbol ester (TPA), interferon, Newcastle Disease Virus, A23187, IL-6, serum, interferon, SV40 large T antigen, PMA, and/or thyroid hormone. Any of the inducible elements described herein may be used with any of the inducers described herein.

Of particular interest are muscle specific promoters. These include the myosin light chain-2 promoter, the α-actin promoter, the troponin 1 promoter; the Na^+/Ca^{2+} exchanger promoter, the dystrophin promoter, the α7 integrin promoter, the brain natriuretic peptide promoter and the αB-crystallin/small heat shock protein promoter, α-myosin heavy chain promoter and the ANF promoter. In some embodiments, the muscle specific promoter is the CK8 promoter. The CK8 promoter has the following sequence (SEQ ID NO. 874):

```
1     CTAGACTAGC ATGCTGCCCA TGTAAGGAGG CAAGGCCTGG GGACACCCGA GATGCCTGGT
61    TATAATTAAC CCAGACATGT GGCTGCCCCC CCCCCCCCAA CACCTGCTGC CTCTAAAAAT
121   AACCCTGCAT GCCATGTTCC CGGCGAAGGG CCAGCTGTCC CCCGCCAGCT AGACTCAGCA
181   CTTAGTTTAG GAACCAGTGA GCAAGTCAGC CCTTGGGGCA GCCCATACAA GGCCATGGGG
241   CTGGGCAAGC TGCACGCCTG GGTCCGGGGT GGGCACGGTG CCCGGGCAAC GAGCTGAAAG
301   CTCATCTGCT CTCAGGGGCC CCTCCCTGGG GACAGCCCCT CCTGGCTAGT CACACCCTGT
361   AGGCTCCTCT ATATAACCCA GGGGCACAGG GGCTGCCCTC ATTCTACCAC CACCTCCACA
421   GCACAGACAG ACACTCAGGA GCCAGCCAGC
```

In some embodiments, the muscle-cell cell specific promoter is a variant of the CK8 promoter, called CK8e. The CK8e promoter has the following sequence (SEQ ID NO. 875):

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  1  TGCCCATGTA AGGAGGCAAG GCCTGGGGAC ACCCGAGATG CCTGGTTATA ATTAACCCAG
 61  ACATGTGGCT GCCCCCCCCC CCCCAACACC TGCTGCCTCT AAAAATAACC CTGCATGCCA
121  TGTTCCCGGC GAAGGGCCAG CTGTCCCCCG CCAGCTAGAC TCAGCACTTA GTTTAGGAAC
181  CAGTGAGCAA GTCAGCCCTT GGGGCAGCCC ATACAAGGCC ATGGGGCTGG GCAAGCTGCA
241  CGCCTGGGTC CGGGGTGGGC ACGGTGCCCG GGCAACGAGC TGAAAGCTCA TCTGCTCTCA
301  GGGGCCCCTC CCTGGGGACA GCCCCTCCTG GCTAGTCACA CCCTGTAGGC TCCTCTATAT
361  AACCCAGGGG CACAGGGGCT GCCCTCATTC TACCACCACC TCCACAGCAC AGACAGACAC
421  TCAGGAGCCA GCCAGC
```

Where a cDNA insert is employed, one will typically desire to include a polyadenylation signal to effect proper polyadenylation of the gene transcript. Any polyadenylation sequence may be employed such as human growth hormone and SV40 polyadenylation signals. Also contemplated as an element of the expression cassette is a terminator. These elements can serve to enhance message levels and to minimize read through from the cassette into other sequences.

Therapeutic Compositions

AAV-Cas9 vectors

In some embodiments, a Cas9 may be packaged into an AAV vector. In some embodiments, the AAV vector is a wildtype AAV vector. In some embodiments, the AAV vector contains one or more mutations. In some embodiments, the AAV vector is isolated or derived from an AAV vector of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV or any combination thereof.

Exemplary AAV-Cas9 vectors contain two ITR (inverted terminal repeat) sequences which flank a central sequence region comprising the Cas9 sequence. In some embodiments, the ITRs are isolated or derived from an AAV vector of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV or any combination thereof. In some embodiments, the ITRs comprise or consist of full-length and/or wildtype sequences for an AAV serotype. In some embodiments, the ITRs comprise or consist of truncated sequences for an AAV serotype. In some embodiments, the ITRs comprise or consist of elongated sequences for an AAV serotype. In some embodiments, the ITRs comprise or consist of sequences comprising a sequence variation compared to a wildtype sequence for the same AAV serotype. In some embodiments, the sequence variation comprises one or more of a substitution, deletion, insertion, inversion, or transposition. In some embodiments, the ITRs comprise or consist of at least 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150 base pairs. In some embodiments, the ITRs

comprise or consist of 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150 base pairs. In some embodiments, the ITRs have a length of 110 ± 10 base pairs. In some embodiments, the ITRs have a length of 120 ± 10 base pairs. In some embodiments, the ITRs have a length of 130 ± 10 base pairs. In some embodiments, the ITRs have a length of 140 ± 10 base pairs. In some embodiments, the ITRs have a length of 150 ± 10 base pairs. In some embodiments, the ITRs have a length of 115, 145, or 141 base pairs.

In some embodiments, the AAV-Cas9 vector may contain one or more nuclear localization signals (NLS). In some embodiments, the AAV-Cas9 vector contains 1, 2, 3, 4, or 5 nuclear localization signals. Exemplary NLS include the c-myc NLS (SEQ ID NO: 884), the SV40 NLS (SEQ ID NO: 885), the hnRNPAI M9 NLS (SEQ ID NO: 886), the nucleoplasmin NLS (SEQ ID NO: 887), the sequence RMRKFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 888) of the IBB domain from importin-alpha, the sequences VSRKRPRP (SEQ ID NO: 889) and PPKKARED (SEQ ID NO: 890) of the myoma T protein, the sequence PQPKKKPL (SEQ ID NO: 891) of human p53, the sequence SALIKKKKKMAP (SEQ ID NO: 892) of mouse c-abl IV, the sequences DRLRR (SEQ ID NO: 893) and KQKKRK (SEQ ID NO: 894) of the influenza virus NS1, the sequence RKLKKKIKKL (SEQ ID NO: 895) of the Hepatitis virus delta antigen and the sequence REKKKFLKRR (SEQ ID NO: 896) of the mouse Mx1 protein. Further acceptable nuclear localization signals include bipartite nuclear localization sequences such as the sequence KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 897) of the human poly(ADP-ribose) polymerase or the sequence RKCLQAGMNLEARKTKK (SEQ ID NO: 898) of the steroid hormone receptors (human) glucocorticoid.

In some embodiments, the AAV-Cas9 vector may comprise additional elements to facilitate packaging of the vector and expression of the Cas9. In some embodiments, the AAV-Cas9 vector may comprise a polyA sequence. In some embodiments, the polyA sequence may be a mini-polyA sequence. In some embodiments, the AAV-CAs9 vector may comprise a transposable element. In some embodiments, the AAV-Cas9 vector may comprise a regulator element. In some embodiments, the regulator element is an activator or a repressor.

In some embodiments, the AAV-Cas9 may contain one or more promoters. In some embodiments, the one or more promoters drive expression of the Cas9. In some embodiments, the one or more promoters are muscle-specific promoters. Exemplary muscle-specific promoters include myosin light chain-2 promoter, the α-actin promoter, the troponin 1

promoter, the Na+/Ca2+ exchanger promoter, the dystrophin promoter, the α7 integrin promoter, the brain natriuretic peptide promoter, the αB-crystallin/small heat shock protein promoter, α-myosin heavy chain promoter, the ANF promoter, the CK8 promoter and the CK8e promoter.

In some embodiments, the AAV-Cas9 vector may be optimized for production in yeast, bacteria, insect cells, or mammalian cells. In some embodiments, the AAV-Cas9 vector may be optimized for expression in human cells. In some embodiments, the AAV-Cas9 vector may be optimized for expression in a bacculovirus expression system.

AAV-sgRNA Vectors

In some embodiments, at least a first sequence encoding a gRNA and a second sequence encoding a gRNA may be packaged into an AAV vector. In some embodiments, at least a first sequence encoding a gRNA, a second sequence encoding a gRNA, and a third sequence encoding a gRNA may be packaged into an AAV vector. In some embodiments, at least a first sequence encoding a gRNA, a second sequence encoding a gRNA, a third sequence encoding a gRNA, and a fourth sequence encoding a gRNA may be packaged into an AAV vector. In some embodiments, at least a first sequence encoding a gRNA, a second sequence encoding a gRNA, a third sequence encoding a gRNA, a fourth sequence encoding a gRNA, and a fifth sequence encoding a gRNA may be packaged into an AAV vector. In some embodiments, a plurality of sequences encoding a gRNA are packaged into an AAV vector. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 sequences encoding a gRNA may be packaged into an AAV vector. In some embodiments, each sequence encoding a gRNA is different. In some embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 of the sequences encoding a gRNA are the same. In some embodiments, all of the sequence encoding a gRNA are the same.

In some embodiments, the AAV vector is a wildtype AAV vector. In some embodiments, the AAV vector contains one or more mutations. In some embodiments, the AAV vector is isolated or derived from an AAV vector of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV or any combination thereof.

Exemplary AAV-sgRNA vectors contain two ITR (inverted terminal repeat) sequences which flank a central sequence region comprising the sgRNA sequences. In some

embodiments, the ITRs are isolated or derived from an AAV vector of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV or any combination thereof. In some embodiments, the ITRs are isolated or derived from an AAV vector of a first serotype and a sequence encoding a capsid protein of the AAV-sgRNA vector is isolated or derived from an AAV vector of a second serotype. In some embodiments, the first serotype and the second serotype are the same. In some embodiments, the first serotype and the second serotype are not the same. In some embodiments, the first serotype is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV. In some embodiments, the second serotype is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV. In some embodiments, the first serotype is AAV2 and the second serotype is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV. In some embodiments, the first serotype is AAV2 and the second serotype is AAV9.

In some embodiments, a first ITR is isolated or derived from an AAV vector of a first serotype, a second ITR is isolated or derived from an AAV vector of a second serotype and a sequence encoding a capsid protein of the AAV-sgRNA vector is isolated or derived from an AAV vector of a third serotype. In some embodiments, the first serotype and the second serotype are the same. In some embodiments, the first serotype and the second serotype are not the same. In some embodiments, the first serotype, the second serotype, and the third serotype are the same. In some embodiments, the first serotype, the second serotype, and the third serotype are not the same. In some embodiments, the first serotype is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, or ovine AAV. In some embodiments, the second serotype is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, or ovine AAV. In some embodiments, the third serotype is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74,

AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, or ovine AAV. In some embodiments, the first serotype is AAV2, the second serotype is AAV4 and the third serotype is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or AAV11. In some embodiments, the first serotype is AAV2, the second serotype is AAV4 and the third serotype is AAV9. Exemplary AAV-sgRNA vectors contain two ITR (inverted terminal repeat) sequences which flank a central sequence region comprising the sgRNA sequences. In some embodiments, the ITRs are isolated or derived from an AAV vector of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV or any combination thereof. In some embodiments, the ITRs comprise or consist of full-length and/or wildtype sequences for an AAV serotype. In some embodiments, the ITRs comprise or consist of truncated sequences for an AAV serotype. In some embodiments, the ITRs comprise or consist of elongated sequences for an AAV serotype. In some embodiments, the ITRs comprise or consist of sequences comprising a sequence variation compared to a wildtype sequence for the same AAV serotype. In some embodiments, the sequence variation comprises one or more of a substitution, deletion, insertion, inversion, or transposition. In some embodiments, the ITRs comprise or consist of at least 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150 base pairs. In some embodiments, the ITRs comprise or consist of 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150 base pairs. In some embodiments, the ITRs have a length of 110 ± 10 base pairs. In some embodiments, the ITRs have a length of 120 ± 10 base pairs. In some embodiments, the ITRs have a length of 130 ± 10 base pairs. In some embodiments, the ITRs have a length of 140 ± 10 base pairs. In some embodiments, the ITRs have a length of 150 ± 10 base pairs. In some embodiments, the ITRs have a length of 115, 145, or 141 base pairs.

In some embodiments, the AAV-sgRNA vector may comprise additional elements to facilitate packaging of the vector and expression of the sgRNA. In some embodiments, the AAV-sgRNA vector may comprise a transposable element. In some embodiments, the AAV-sgRNA vector may comprise a regulatory element. In some embodiments, the regulatory element comprises an activator or a repressor. In some embodiments, the AAV-sgRNA

sequence may comprise a non-functional or "stuffer" sequence. Exemplary stuffer sequences of the disclosure may have some (a non-zero percentage of) identity or homology to a genomic sequence of a mammal (including a human). Alternatively, exemplary stuffer sequences of the disclosure may have no identity or homology to a genomic sequence of a mammal (including a human). Exemplary stuffer sequences of the disclosure may comprise or consist of naturally occurring non-coding sequences or sequences that are neither transcribed nor translated following administration of the AAV vector to a subject.

In some embodiments, the AAV-sgRNA vector may be optimized for production in yeast, bacteria, insect cells, or mammalian cells. In some embodiments, the AAV-sgRNA vector may be optimized for expression in human cells. In some embodiments, the AAV-Cas9 vector may be optimized for expression in a bacculovirus expression system.

In some embodiments, the AAV-sgRNA vector comprises at least one promoter. In some embodiments, the AAV-sgRNA vector comprises at least two promoters. In some embodiments, the AAV-sgRNA vector comprises at least three promoters. In some embodiments, the AAV-sgRNA vector comprises at least four promoters. In some embodiments, the AAV-sgRNA vector comprises at least five promoters. Exemplary promoters include, for example, immunoglobulin light chain, immunoglobulin heavy chain, T-cell receptor, HLA DQ a and/or DQ β, β-interferon, interleukin-2, interleukin-2 receptor, MHC class II 5, MHC class II HLA-Dra, β-Actin, muscle creatine kinase (MCK), prealbumin (transthyretin), elastase I, metallothionein (MTII), collagenase, albumin, α-fetoprotein, t-globin, β-globin, c-fos, c-HA-*ras,* insulin, neural cell adhesion molecule (NCAM), α_1-antitrypain, H2B (TH2B) histone, mouse and/or type I collagen, glucose-regulated proteins (GRP94 and GRP78), rat growth hormone, human serum amyloid A (SAA), troponin I (TN I), platelet-derived growth factor (PDGF), duchenne muscular dystrophy, SV40, polyoma, retroviruses, papilloma virus, hepatitis B virus, human immunodeficiency virus, cytomegalovirus (CMV), and gibbon ape leukemia virus. Further exemplary promoters include the U6 promoter, the H1 promoter, and the 7SK promoter.

In some embodiments, the sequence encoding the gRNA or the genomic target sequence comprises a sequence selected from SEQ ID NOs. 60-705, 712-862, and 947-2377.

Pharmaceutical Compositions and Delivery Methods

Also provided herein are compositions comprising one or more vectors and/or nucleic acids of the disclosure. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

For clinical applications, pharmaceutical compositions are prepared in a form appropriate for the intended application. Generally, this entails preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

Appropriate salts and buffers are used to render drugs, proteins or delivery vectors stable and allow for uptake by target cells. Aqueous compositions of the present disclosure comprise an effective amount of the drug, vector or proteins, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. The phrase "pharmaceutically or pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" includes solvents, buffers, solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like acceptable for use in formulating pharmaceuticals, such as pharmaceuticals suitable for administration to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Any conventional media or agent that is not incompatible with the active ingredients of the present disclosure, its use in therapeutic compositions may be used. Supplementary active ingredients also can be incorporated into the compositions, provided they do not inactivate the vectors or cells of the compositions.

In some embodiments, the active compositions of the present disclosure may include classic pharmaceutical preparations. Administration of these compositions according to the present disclosure may be via any common route so long as the target tissue is available via that route, but generally including systemic administration. This includes oral, nasal, or buccal. Alternatively, administration may be by intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or by direct injection into muscle tissue. Such compositions would normally be administered as pharmaceutically acceptable compositions, as described *supra*.

The active compounds may also be administered parenterally or intraperitoneally. By way of illustration, solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include, for example, sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Generally, these preparations are sterile and fluid to the extent that easy injectability exists. Preparations should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Appropriate solvents or dispersion media may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and 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 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 the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions may be prepared by incorporating the active compounds in an appropriate amount into a solvent along with any other ingredients (for example as enumerated above) as desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the desired other ingredients, *e.g.*, as enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation include vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient(s) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In some embodiments, the compositions of the present disclosure are formulated in a neutral or salt form. Pharmaceutically-acceptable salts include, for example, acid addition salts (formed with the free amino groups of the protein) derived from inorganic acids (*e.g.*, hydrochloric or phosphoric acids, or from organic acids (*e.g.*, acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups of the protein can also be derived from inorganic bases (*e.g.*, sodium, potassium, ammonium, calcium, or ferric hydroxides) or from organic bases (*e.g.*, isopropylamine, trimethylamine, histidine, procaine) and the like.

Upon formulation, solutions are preferably administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations may easily be administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution generally is suitably buffered and the liquid diluent first rendered isotonic for example with sufficient saline or glucose. Such aqueous solutions may be used, for example, for intravenous, intramuscular, subcutaneous and intraperitoneal administration. Preferably, sterile aqueous media are employed as is known to those of skill in the art, particularly in light of the present disclosure. By way of illustration, a single dose may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

In some embodiments, the Cas9 or Cpf1 and gRNAs described herein may be delivered to the patient using adoptive cell transfer (ACT). In adoptive cell transfer, one or more expression constructs are provided ex vivo to cells which have originated from the patient (autologous) or from one or more individual(s) other than the patient (allogeneic). The cells are subsequently introduced or reintroduced into the patient. Thus, in some embodiments, one or more nucleic acids encoding Cas9 or Cpf1 and a guide RNA that targets a dystrophin splice site are provided to a cell ex vivo before the cell is introduced or reintroduced to a patient.

Cells and Cell Compositions

Also provided is a cell comprising one or more nucleic acids of the disclosure. In some embodiments, the cell is a human cell. In some embodiments, the cell is a muscle cell or satellite cell. In some embodiments, the cell is an induced pluripotent stem (iPS) cell. In some embodiments, the cell is a cardiomyocyte. In some embodiments, the cell (e.g., a cardiomyocyte) is derived from an iPS cell.

Also provided is a cell comprising a composition comprising one or more vectors of the disclosure. In some embodiments, the cell is a human cell. In some embodiments, the cell is a muscle cell or satellite cell. In some embodiments, the cell is an induced pluripotent stem (iPS) cell. In some embodiments, the cell is a cardiomyocyte. In some embodiments, the cell (e.g., a cardiomyocyte) is derived from an iPS cell.

Also provided is a cell produced by one or more methods of the disclosure. In some embodiments, the cell is a human cell. In some embodiments, the cell is a muscle cell or satellite cell. In some embodiments, the cell is an induced pluripotent stem (iPS) cell. In some embodiments, the cell is a cardiomyocyte. In some embodiments, the cell (e.g., a cardiomyocyte) is derived from an iPS cell.

Also provided is a composition comprising a cell comprising one or more nucleic acids of the disclosure. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

Therapeutic Methods and Uses

The disclosure also provides methods for editing a dystrophin gene, such as a mutant dystrophin gene, in a cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a muscle cell or satellite cell. In some embodiments, the cell is an induced pluripotent stem (iPS) cell. In some embodiments, the cell is a cardiomyocyte. In some embodiments, the cell (e.g., a cardiomyocyte) is derived from an iPS cell.

In some embodiments, the disclosure provides a method for editing a mutant dystrophin gene in a cardiomyocyte, the method comprising contacting the cardiomyocyte with a Cas9 nuclease, or a sequence encoding a Cas9 nuclease, and a gRNA, or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene. The mutant dystrophin gene may comprise one or more mutations, such as a point mutation (e.g., a pseudo-exon mutation), a deletion, and/or a duplication mutation. A deletion may be a deletion of at least 20, at least 50, at least 100, at least 500, at least 1000, at least 3000

nucleotides, at least 5000 nucleotides or at least 10,000 nucleotides. In some embodiments, the deletion comprises a deletion of one or more exons, one or more introns, or at least a portion of one intron and one exon.

In some embodiments, the disclosure provides a method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising administering to the subject a Cas9 nuclease, or a sequence encoding a Cas9 nuclease, and a gRNA, or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene, wherein the administering restores dystrophin expression in at least 10% of the subject's cardiomyocytes. In some embodiments, the administering restores dystrophin expression in at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of the subject's cardiomyocytes. The average human heart has approximately 2 to 3 billion cardiomyocytes. Accordingly, in some embodiments, the administering restores dystrophin expression in at least 2×10^8, at least 3×10^8, at least 4×10^8, at least 5×10^8, at least 6×10^8, at least 7×10^8, at least 8×10^8, at least 9×10^8, at least 10×10^8, at least 11×10^8, at least 12×10^8, at least 13×10^8, at least 14×10^8, at least 15×10^8, at least 16×10^8, at least 17×10^8, at least 18×10^8, at least 19×10^8, at least 20×10^8, at least 21×10^8, at least 22×10^8, at least 23×10^8, at least 24×10^8, at least 25×10^8, at least 26×10^8, at least 27×10^8, at least 28×10^8, at least 29×10^8, at least 30×10^8 of the subject's cardiomyocytes. In some embodiments, the subject suffers from dilated cardiomyopathy. In some embodiments, the administering at least partially rescues cardiac contractility, or completely rescues cardiac contractility.

In some embodiments, a method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, is provided, the method comprising contacting an induced pluripotent stem cell (iPSC) with a Cas9 nuclease or a sequence encoding a Cas9 nuclease, and a gRNA or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene; differentiating the iPSC into a cardiomyocyte; and administering the cardiomyocyte to the subject. In some embodiments, at least 1×10^3, at least 1×10^4, at least 1×10^5, at least 1×10^6, at least 1×10^7 or at least 1×10^8 cardiomyocytes are administered to the patient.

The gRNA may target, for example a splice donor or splice acceptor site of exon 51, 45, 53, 44, 46, 52, 50, 43, 6, 7, 8, or 55 of the cardiomyocyte dystrophin gene. In some embodiments, the gRNA or the genomic targeting sequence has a sequence of any one of SEQ ID NOs. 60-705, 712-862, 947-2377. The cas9 nuclease may be isolated or derived from, for example, a S. pyogenes (spCas9) or a S. aureus cas9 (saCas9).

In some embodiments, a vector comprising the gRNA, or a sequence encoding the gRNA, is contacted with the cardiomyocyte. The vector may be, for example, non-viral vector such as a plasmid or a nanoparticle. In some embodiments, the vector may be a viral vector, such as an adeno-associated viral (AAV) vector. In some embodiments, the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV.

In some embodiments, a single vector comprising the Cas9 nuclease, or a sequence encoding the Cas9 nuclease, and the gRNA, or a sequence encoding the gRNA, are contacted with the cardiomyocyte. In other embodiments, a first vector comprising the Cas9 nuclease, or a sequence encoding the Cas9 nuclease, and a second vector comprising the gRNA or a sequence encoding the gRNA, are contacted with the cardiomyocyte. The first and second vector may be the same or may be different. For example, the first vector and the second vector may both be AAVs, or the first vector may be an AAV and the second vector may be a plasmid.

Also provided is a method for correcting a dystrophin defect, the method comprising contacting a cell with one or more compositions of the disclosure under conditions suitable for expression of the guide RNA, the Cas9 protein or a nuclease domain thereof, wherein the guide RNA forms a complex with the Cas9 protein or the nuclease domain thereof to form at least one guide RNA-Cas9 complex, wherein the at least one guide RNA-Cas9 complex disrupts a dystrophin splice site and induces selective skipping of a DMD exon and/or reframing. In some embodiments, the at least one guide RNA-Cas9 complex disrupts a dystrophin splice site and induces a reframing of a dystrophin reading frame. In some embodiments, the at least one guide RNA-Cas9 complex disrupts a dystrophin splice site and produces an insertion which restores the dystrophin protein reading frame. In some embodiments, the insertion comprises an insertion of a single adenosine.

Also provided is a method for inducing selective skipping and/or reframing of a DMD exon, the method comprising contacting a cell with one or more compositions of the disclosure under conditions suitable for expression of the guide RNA and the Cas9 protein or a nuclease domain thereof, wherein the guide RNA and the second guide RNA form a complex with the Cas9 protein or the nuclease domain thereof to form at least one guide RNA-Cas9 complex, wherein the at least one guide RNA-Cas9 complex disrupts a dystrophin splice site and induces selective skipping and/or reframing of a DMD exon.

Also provided is a method for inducing a reframing event in the dystrophin reading frame, the method comprising contacting a cell with one or more compositions of the disclosure

under conditions suitable for expression of the guide RNA and the Cas9 protein or a nuclease domain thereof, wherein the guide RNA forms a complex with the Cas9 protein or the nuclease domain thereof to form at least one guide RNA-Cas9 complex, wherein the guide RNA-Cas9 complex disrupts a dystrophin splice site and induces selective skipping and/or reframing of a DMD exon. In some embodiments, the at least one guide RNA-Cas9 complex disrupts a dystrophin splice site and induces selective skipping and/or reframing of exon 51 of a human DMD gene.

Also provided is a method of treating or preventing muscular dystrophy in a subject in need thereof comprising administering to the subject a therapeutically effective amount of one or more compositions of the disclosure. In some embodiments, the composition is administered locally. In some embodiments, the composition is administered directly to a muscle tissue. In some embodiments, the composition is administered by an intramuscular infusion or injection. In some embodiments, the muscle tissue comprises a tibialis anterior tissue, a quadriceps tissue, a soleus tissue, a diaphragm tissue, or a heart tissue. In some embodiments, the composition is administered by an intra-cardiac injection. In some embodiments, the composition is administered systemically. In some embodiments, the composition is administered by an intravenous infusion or injection. In some embodiments, following administration of the composition, the subject exhibits normal dystrophin-positive myofibers, and mosaic dystrophin-positive myofibers containing centralized nuclei, or a combination thereof. In some embodiments, following administration of the composition, the subject exhibits an emergence or an increase in a level of abundance of normal dystrophin-positive myofibers when compared to an absence or a level of abundance of normal dystrophin-positive myofibers prior to administration of the composition. In some embodiments, following administration of the composition, the subject exhibits an emergence or an increase in a level of abundance of mosaic dystrophin-positive myofibers containing centralized nuclei when compared to an absence or an level of abundance of mosaic dystrophin-positive myofibers containing centralized nuclei prior to administration of the composition. In some embodiments, following administration of the composition, the subject exhibits a decreased serum CK level when compared to a serum CK level prior to administration of the composition. In some embodiment, following administration of the composition, the subject exhibits improved grip strength when compared to a grip strength prior to administration of the composition. In some embodiments, the subject is a neonate, an infant, a child, a young adult, or an adult. In some embodiments, the subject has muscular dystrophy. In some embodiments, the subject is a genetic carrier for muscular dystrophy. In some embodiments, the subject is male. In some embodiments, the subject is

female. In some embodiments, the subject appears to be asymptomatic and a genetic diagnosis reveals a mutation in one or both copies of a *DMD* gene that impairs function of the *DMD* gene product. In some embodiments, the subject presents an early sign or symptom of muscular dystrophy. In some embodiments, the early sign or symptom of muscular dystrophy comprises loss of muscle mass or proximal muscle weakness. In some embodiments, the loss of muscle mass or proximal muscle weakness occurs in one or both leg(s) and/or a pelvis, followed by one or more upper body muscle(s). In some embodiments, the early sign or symptom of muscular dystrophy further comprises pseudohypertrophy, low endurance, difficulty standing, difficulty walking, difficulty ascending a staircase or a combination thereof. In some embodiments, the subject presents a progressive sign or symptom of muscular dystrophy. In some embodiments, the progressive sign or symptom of muscular dystrophy comprises muscle tissue wasting, replacement of muscle tissue with fat, or replacement of muscle tissue with fibrotic tissue. In some embodiments, the subject presents a later sign or symptom of muscular dystrophy. In some embodiments, the later sign or symptom of muscular dystrophy comprises abnormal bone development, curvature of the spine, loss of movement, and paralysis. In some embodiments, the subject presents a neurological sign or symptom of muscular dystrophy. In some embodiments, the neurological sign or symptom of muscular dystrophy comprises intellectual impairment and paralysis. In some embodiments, administration of the composition occurs prior to the subject presenting one or more progressive, later or neurological signs or symptoms of muscular dystrophy. In some embodiments, the subject greater than 18 years old, greater than 25 years old, or greater than 30 years old. In some embodiments, the subject is less than 18 years old, less than 16 years old, less than 12 years old, less than 10 years old, less than 5 years old, or less than 2 years old. Also provided is the use of a therapeutically-effective amount of one or more compositions of the disclosure for treating muscular dystrophy in a subject in need thereof.

Delivery Vectors

There are a number of ways in which expression vectors may be introduced into cells. In certain embodiments, the expression construct comprises a virus or engineered construct derived from a viral genome. The ability of certain viruses to enter cells via receptor-mediated endocytosis, to integrate into host cell genome and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign genes into mammalian cells. These have a relatively low capacity for foreign DNA sequences and have a restricted host spectrum. Furthermore, their oncogenic potential and cytopathic effects in permissive cells

raise safety concerns. They can accommodate only up to 8 kB of foreign genetic material but can be readily introduced in a variety of cell lines and laboratory animals.

One of the preferred methods for *in vivo* delivery involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express an antisense polynucleotide that has been cloned therein. In this context, expression does not require that the gene product be synthesized.

The expression vector comprises a genetically engineered form of adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kB, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kB. In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target cell range and high infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are *cis* elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome contain different transcription units that are divided by the onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off. The products of the late genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP, (located at 16.8 m.u.) is particularly efficient during the late phase of infection, and all the mRNAs issued from this promoter possess a 5'-tripartite leader (TPL) sequence which makes them preferred mRNAs for translation. In one system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible recombination between two proviral vectors, wild-type adenovirus may be

generated from this process. Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins. Since the E3 region is dispensable from the adenovirus genome, the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions. In nature, adenovirus can package approximately 105% of the wild-type genome, providing capacity for about 2 extra kb of DNA. Combined with the approximately 5.5 kb of DNA that is replaceable in the E1 and E3 regions, the maximum capacity of the current adenovirus vector is under 7.5 kb, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-borne cytotoxicity. Also, the replication deficiency of the E1-deleted virus is incomplete.

Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, *e.g.*, Vero cells or other monkey embryonic mesenchymal or epithelial cells. As stated above, the preferred helper cell line is 293.

Improved methods for culturing 293 cells and propagating adenovirus are known in the art. In one format, natural cell aggregates are grown by inoculating individual cells into 1 liter siliconized spinner flasks (Techne, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and adenovirus added at an MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

The adenoviruses of the disclosure are replication defective, or at least conditionally replication defective. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain the conditional replication-defective adenovirus vector for use in the present disclosure.

As stated above, the typical vector according to the present disclosure is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors, or in the E4 region where a helper cell line or helper virus complements the E4 defect.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, *e.g.*, 10^9-10^{12} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus, demonstrating their safety and therapeutic potential as *in vivo* gene transfer vectors.

Adenovirus vectors have been used in eukaryotic gene expression and vaccine development. Animal studies suggested that recombinant adenovirus could be used for gene therapy. Studies in administering recombinant adenovirus to different tissues include trachea instillation, muscle injection, peripheral intravenous injections and stereotactic inoculation into the brain.

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription. The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present

at the 5' and 3' ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome.

In order to construct a retroviral vector, a nucleic acid encoding a gene of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed. When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media. The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells.

A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes via sialoglycoprotein receptors.

A different approach to targeting of recombinant retroviruses may be used, in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor are used. The antibodies are coupled via the biotin components by using streptavidin. Using antibodies against major histocompatibility complex class I and class II antigens, it has been demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro*.

There are certain limitations to the use of retrovirus vectors in all aspects of the present disclosure. For example, retrovirus vectors usually integrate into random sites in the cell genome. This can lead to insertional mutagenesis through the interruption of host genes or through the insertion of viral regulatory sequences that can interfere with the function of flanking genes. Another concern with the use of defective retrovirus vectors is the potential appearance of wild-type replication-competent virus in the packaging cells. This can result from recombination events in which the intact-sequence from the recombinant virus inserts upstream from the gag, pol, env sequence integrated in the host cell genome. However, new

packaging cell lines are now available that should greatly decrease the likelihood of recombination (see, for example, Markowitz *et al.*, 1988; Hersdorffer *et al.*, 1990).

Other viral vectors may be employed as expression constructs in the present disclosure. Vectors derived from viruses such as vaccinia virus adeno-associated virus (AAV) and herpesviruses may be employed. They offer several attractive features for various mammalian cells.

In embodiments, the AAV vector is replication-defective or conditionally replication defective. In embodiments, the AAV vector is a recombinant AAV vector. In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV vector of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, or ovine AAV or any combination thereof.

In some embodiments, a single viral vector is used to deliver a nucleic acid encoding a Cas9 or a Cpf1 and at least one gRNA to a cell. In some embodiments, Cas9 or Cpf1 is provided to a cell using a first viral vector and at least one gRNA is provided to the cell using a second viral vector.

In some embodiments, a single viral vector is used to deliver a nucleic acid encoding Cas9 or Cpf1 and at least one gRNA to a cell. In some embodiments, Cas9 or Cpf1 is provided to a cell using a first viral vector and at least one gRNA is provided to the cell using a second viral vector. In order to effect expression of sense or antisense gene constructs, the expression construct must be delivered into a cell. The cell may be a muscle cell, a satellite cell, a mesangioblast, a bone marrow derived cell, a stromal cell or a mesenchymal stem cell. In embodiments, the cell is a cardiac muscle cell, a skeletal muscle cell, or a smooth muscle cell. In embodiments, the cell is a cell in the tibialis anterior, quadriceps, soleus, diaphragm or heart. In some embodiments, the cell is an induced pluripotent stem cell (iPSC) or inner cell mass cell (iCM). In further embodiments, the cell is a human iPSC or a human iCM. In some embodiments, human iPSCs or human iCMs of the disclosure may be derived from a cultured stem cell line, an adult stem cell, a placental stem cell, or from another source of adult or embryonic stem cells that does not require the destruction of a human embryo. Delivery to a cell may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. One mechanism for delivery is via viral infection where the expression construct is encapsidated in an infectious viral particle.

Several non-viral methods for the transfer of expression constructs into cultured mammalian cells also are contemplated by the present disclosure. These include calcium phosphate precipitation, DEAE-dextran, electroporation, direct microinjection, DNA-loaded liposomes and lipofectamine-DNA complexes, cell sonication, gene bombardment using high velocity microprojectiles, and receptor-mediated transfection. Some of these techniques may be successfully adapted for *in vivo* or *ex vivo* use.

Once the expression construct has been delivered into the cell the nucleic acid encoding the gene of interest may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the gene may be stably integrated into the genome of the cell. This integration may be in the cognate location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In yet another embodiment, the expression construct may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well.

In still another embodiment for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them. Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force. The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

In some embodiments, the expression construct is delivered directly to the liver, skin, and/or muscle tissue of a subject. This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, *i.e.*, *ex vivo* treatment. Again, DNA encoding a particular gene may be delivered via this method and still be incorporated by the present disclosure.

In a further embodiment, the expression construct may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers. Also contemplated are lipofectamine-DNA complexes.

Liposome-mediated nucleic acid delivery and expression of foreign DNA *in vitro* has been very successful. A reagent known as Lipofectamine 2000™ is widely used and commercially available.

In certain embodiments, the liposome may be complexed with a hemagglutinating virus (HVJ) to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA. In other embodiments, the liposome may be complexed or employed in conjunction with nuclear non-histone chromosomal proteins (HMG-1). In yet further embodiments, the liposome may be complexed or employed in conjunction with both HVJ and HMG-1. In that such expression constructs have been successfully employed in transfer and expression of nucleic acid *in vitro* and *in vivo*, then they are applicable for the present disclosure. Where a bacterial promoter is employed in the DNA construct, it also will be desirable to include within the liposome an appropriate bacterial polymerase.

Other expression constructs which can be employed to deliver a nucleic acid encoding a particular gene into cells are receptor-mediated delivery vehicles. These take advantage of the selective uptake of macromolecules by receptor-mediated endocytosis in almost all eukaryotic cells. Because of the cell type-specific distribution of various receptors, the delivery can be highly specific.

Receptor-mediated gene targeting vehicles generally consist of two components: a cell receptor-specific ligand and a DNA-binding agent. Several ligands have been used for receptor-mediated gene transfer. The most extensively characterized ligands are asialoorosomucoid (ASOR) and transferrin. A synthetic neoglycoprotein, which recognizes the same receptor as ASOR, has been used as a gene delivery vehicle and epidermal growth factor (EGF) has also been used to deliver genes to squamous carcinoma cells.

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is a recessive X-linked form of muscular dystrophy, affecting around 1 in 5000 boys, which results in muscle degeneration and premature death. The disorder is caused by a mutation in the gene dystrophin (see GenBank Accession NO. NC_000023.11), located on the human X chromosome, which codes for the protein dystrophin (GenBank Accession No. AAA53189; SEQ ID NO: 5).

In humans, dystrophin mRNA contains 79 exons. Dystrophin mRNA is known to be alternatively spliced, resulting in various isoforms. Exemplary dystrophin isoforms are listed in Table 1.

The murine dystrophin protein has the following amino acid sequence (Uniprot Accession No. P11531, SEQ. ID. NO: 869):

```
   1 MWWVDCYRDV KKTTKWNASK GKHDNSDDGK RDGTGKKKGS TRVHANNVNK ARVKNNVDVN
  61 GSTDVDGNHK TGWNHWVKNV MKTMAGTNSK SWVRSTRNYV NVNTSSWSDG ANAHSHRDDW
 121 NSVVSHSATR HANAKCGKDD VATTYDKKSM YTSVVSAVMR TSSKVTRHHH MHYSTVSAGY
 181 TSSSKRKSYA TAAYVATSDS TSYSHARDKS DSSMTVNDSY TAVSWSADTR AGSNDVVKHA
 241 HGMMDTSHGV GNVGSVGKGK SDAVMNNSRW CRVASMKSKH KVMDNKKDDW TKTRTKKMGD
 301 DKCVHKVDVR VNSTHMVVVV DSSGDHATAA KVGDRWANCR WTDRWVDKWH TCSTWSKDAM
 361 KNTSGKDNMM SSHKSTKDKK KTMKSSNDSA KNKSVTKMWM NARWDNTKKS SASAVTTTST
 421 TTVMTVTMVT TRMVKHAKKR TVDSRKRDVD THSWTRSAVS SAVYRKGNSD KVNAARKAKR
 481 KDASRSAAVM ANGVNASRAS NSRWTCSRVN WYTNTYNMTT TANKTSTTST AKSKCKDVNR
 541 SAKSKKGGMD ADVATNHNHD GVRAKKTDTM RYTMSSRTWS SKSVYSVTYM RGKASSKNGN
 601 YSDTVKMAKK ASCKYSGHWK KSSVSCKHMN KRKNHKTKWM AVDVKWAGDA KKKCRVGDTS
 661 NSVNGGKKSA ASRTRNTWDH CRVYTRKAKA GDKTVSKDSM HWMTAYRDYK TDTAVMKRAK
 721 AKTKVKTTVN SVAHASAAKK TTTNYWCTRN GKCKTVWACW HSYKANKWNV KKTMNVAGTV
 781 SNMHHSNNRA TTDGGVMDNT NSRWRHAVRK KSSAKSHSDK AAYTDKVDAA MAKSDTSHSM
 841 KKHNGKDANR VSDVAKKDVS MKRKANRSKM DVKMHATKSV VSSHCVNYKS SVKSVMVKTG
 901 RVKKTNKDRV TAKHYNGAKV TRKKCKSRKM RKMNVTWAAT DTTKRSAVGM SNDSVAWGKA
 961 TKKKAHKSVT GSKMVGKKTV DKSNSNWAVT SRVWNYKHMT DNTKWHADDS KKKKDKRKAM
1021 NDMRKVDSTR DAAKMANRGD HCRKVVSNRR AASHRKTGKA SKNSDKAGVN KDNKDMSDNG
1081 TVNRGDNRTD RKRKKTKHNA KDRSRRKKAS HWYYKRADDK CDKKASRDRK KDRKKKNAVR
1141 RAGSNGAAMA VTSKRWRSNA RRNAHTHTMV VTTDMDVSYV STYTSHASVD HNTCAKDDKS
1201 KNKDNSGRDH KKKTAASATS MKVKVAVAMD GKHRMYKRGR DRSVKWRHHY DMKVNWNVKK
1261 TNNWHAKYKW YKDGGRAVVR TNATGSSKTD VNKGSSRWHD CKARRKRKNV SRDNVWADNA
1321 TGDKVKARGK NTGGAVVSAR DKKKKTNWKV SRAKGVHKDR DHWSRNYNSA GDKVTVHGKA
1381 DVRSKGHYKK STVKRKDRSW AVNHRRTKDR AGSTTGASAS TVTVTSVVTK TVSKMSSVAA
1441 DNRAWTTDWS DRVKSRVMVG DDNMKKATDR RTAANKNKTS NARTTDRRWD VNRRNMKDST
1501 WAKAVGVRGK DSWKGHTVDA KKTTKAKDRR SVDVANDAKR DYSADDTRKV HMTNNTSWGN
1561 HKRVSAATHR DKSWTATTAN VDASRKKDSR GVRMKWDGTH TDYHNDNGKR SGSDARRDNM
1621 NKWSKKSNRS HASSDWKRHS VWKDDSRAGG DAVKNDHRAK RKTKVMSTTV RTGKYRRANV
1681 TRRKAVNAWD KNRSADWRKD ARAADDKRAV KGSWVGDDSD HKVKARGAKN VNRVNDAHTT
1741 GSYNSTDNTR WRVAVDRVRH AHRDGASHST SVGWRASNKV YYNHTTTCWD HKMTYSADNN
1801 VRSAYRTAMK RRKACDSSAA CDADHNKNDM DNCTTYDRHN NVNVCVDMCN WNVYDTGRTG
1861 RRVSKTGSCK AHDKYRYKVA SSTGCDRRGH DSRGVASGGS NSVRSCANNK AADWMRSMVW
1921 VHRVAAATAK HAKCNCKCGR YRSKHNYDCS CSGRVAKGHK MHYMVYCTTT SGDVRDAKVK
1981 NKRTKRYAKH RMGYVTVGDN MTVTNWVDSA ASSSHDDTHS RHYASRAMNS NGSYNDSSNS
2041 DDHHYCSNDS SRSASSRGRA DNRNAYDRKH HKGSSMMTSS RDAAAKRHKG RARMDHNKSH
2101 RRAAKVNGTT VSSSTSRSDS SMRVVGSTSS MGDSDTSTGV MNNSSSRGRN AGKMRDTM
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Dystrophin is an important component within muscle tissue that provides structural stability to the dystroglycan complex (DGC) of the cell membrane. While both sexes can carry the mutation, females are rarely affected with the skeletal muscle form of the disease.

Mutations vary in nature and frequency. Large genetic deletions are found in about 60-70% of cases, large duplications are found in about 10% of cases, and point mutants or other small changes account for about 15-30% of cases. Bladen *et al.* (2015), who examined some 7000 mutations, catalogued a total of 5,682 large mutations (80% of total mutations), of which 4,894 (86%) were deletions (1 exon or larger) and 784 (14%) were duplications (1 exon or larger). There were 1,445 small mutations (smaller than 1 exon, 20% of all mutations), of which 358 (25%) were small deletions and 132 (9%) small insertions, while 199 (14%) affected the splice sites. Point mutations totaled 756 (52% of small mutations) with 726 (50%) nonsense mutations and 30 (2%) missense mutations. Finally, 22 (0.3%) mid-intronic mutations were observed. In addition, mutations were identified within the database that would potentially benefit from novel genetic therapies for DMD including stop codon read-through therapies (10% of total mutations) and exon skipping therapy (80% of deletions and 55% of total mutations).

DMD Subject Characteristics and Clinical Presentation. Symptoms usually appear in boys between the ages of 2 and 3 and may be visible in early infancy. Even though symptoms do not appear until early infancy, laboratory testing can identify children who carry the active mutation at birth. Progressive proximal muscle weakness of the legs and pelvis associated with loss of muscle mass is observed first. Eventually this weakness spreads to the arms, neck, and other areas. Early signs may include pseudohypertrophy (enlargement of calf and deltoid muscles), low endurance, and difficulties in standing unaided or inability to ascend staircases. As the condition progresses, muscle tissue experiences wasting and is eventually replaced by fat and fibrotic tissue (fibrosis). By age 10, braces may be required to aid in walking but most patients are wheelchair dependent by age 12. Later symptoms may include abnormal bone development that lead to skeletal deformities, including curvature of the spine. Due to progressive deterioration of muscle, loss of movement occurs, eventually leading to paralysis. Intellectual impairment may or may not be present but if present, does not progressively worsen as the child ages. The average life expectancy for males afflicted with DMD is around 25.

The main symptom of Duchenne muscular dystrophy, a progressive neuromuscular disorder, is muscle weakness associated with muscle wasting with the voluntary muscles being

first affected, especially those of the hips, pelvic area, thighs, shoulders, and calves. Muscle weakness also occurs later, in the arms, neck, and other areas. Calves are often enlarged. Symptoms usually appear before age 6 and may appear in early infancy. Other physical symptoms are:

1. Awkward manner of walking, stepping, or running – (patients tend to walk on their forefeet, because of an increased calf muscle tone. Also, toe walking is a compensatory adaptation to knee extensor weakness.)
2. Frequent falls.
3. Fatigue.
4. Difficulty with motor skills (running, hopping, jumping).
5. Lumbar hyperlordosis, possibly leading to shortening of the hip-flexor muscles. This has an effect on overall posture and a manner of walking, stepping, or running.
6. Muscle contractures of Achilles tendon and hamstrings impair functionality because the muscle fibers shorten and fibrose in connective tissue.
7. Progressive difficulty walking.
8. Muscle fiber deformities.
9. Pseudohypertrophy (enlarging) of tongue and calf muscles. The muscle tissue is eventually replaced by fat and connective tissue, hence the term pseudohypertrophy.
10. Higher risk of neurobehavioral disorders (*e.g.*, ADHD), learning disorders (dyslexia), and non-progressive weaknesses in specific cognitive skills (in particular short-term verbal memory), which are believed to be the result of absent or dysfunctional dystrophin in the brain.
11. Eventual loss of ability to walk (usually by the age of 12).
12. Skeletal deformities (including scoliosis in some cases).
13. Trouble getting up from lying or sitting position.

The condition can often be observed clinically from the moment the patient takes his first steps, and the ability to walk usually completely disintegrates between the time the boy is 9 to 12 years of age. Most men affected with DMD become essentially "paralyzed from the neck down" by the age of 21. Muscle wasting begins in the legs and pelvis, then progresses to the muscles of the shoulders and neck, followed by loss of arm muscles and respiratory muscles. Calf muscle enlargement (pseudohypertrophy) is quite obvious. Cardiomyopathy

particularly (dilated cardiomyopathy) is common, but the development of congestive heart failure or arrhythmia (irregular heartbeat) is only occasional.

A positive Gowers' sign reflects the more severe impairment of the lower extremities muscles. The child helps himself to get up with upper extremities: first by rising to stand on his arms and knees, and then "walking" his hands up his legs to stand upright. Affected children usually tire more easily and have less overall strength than their peers. Creatine kinase (CPK-MM) levels in the bloodstream are extremely high. An electromyography (EMG) shows that weakness is caused by destruction of muscle tissue rather than by damage to nerves. Genetic testing can reveal genetic errors in the Xp21 gene. A muscle biopsy (immunohistochemistry or immunoblotting) or genetic test (blood test) confirms the absence of dystrophin, although improvements in genetic testing often make this unnecessary.

DMD patients may suffer from:

1. Abnormal heart muscle (cardiomyopathy).
2. Congestive heart failure or irregular heart rhythm (arrhythmia).
3. Deformities of the chest and back (scoliosis).
4. Enlarged muscles of the calves, buttocks, and shoulders (around age 4 or 5). These muscles are eventually replaced by fat and connective tissue (pseudohypertrophy).
5. Loss of muscle mass (atrophy).
6. Muscle contractures in the heels, legs.
7. Muscle deformities.
8. Respiratory disorders, including pneumonia and swallowing with food or fluid passing into the lungs (in late stages of the disease).

Duchenne muscular dystrophy (DMD) is caused by a mutation of the dystrophin gene at locus Xp21, located on the short arm of the X chromosome. Dystrophin is responsible for connecting the cytoskeleton of each muscle fiber to the underlying basal lamina (extracellular matrix), through a protein complex containing many subunits. The absence of dystrophin permits excess calcium to penetrate the sarcolemma (the cell membrane). Alterations in calcium and signaling pathways cause water to enter into the mitochondria, which then burst.

In skeletal muscle dystrophy, mitochondrial dysfunction gives rise to an amplification of stress-induced cytosolic calcium signals and an amplification of stress-induced reactive-oxygen species (ROS) production. In a complex cascading process that involves several

pathways and is not clearly understood, increased oxidative stress within the cell damages the sarcolemma and eventually results in the death of the cell. Muscle fibers undergo necrosis and are ultimately replaced with adipose and connective tissue.

DMD is inherited in an X-linked recessive pattern. Females will typically be carriers for the disease while males will be affected. Typically, a female carrier will be unaware they carry a mutation until they have an affected son. The son of a carrier mother has a 50% chance of inheriting the defective gene from his mother. The daughter of a carrier mother has a 50% chance of being a carrier and a 50% chance of having two normal copies of the gene. In all cases, an unaffected father will either pass a normal Y to his son or a normal X to his daughter. Female carriers of an X-linked recessive condition, such as DMD, can show symptoms depending on their pattern of X-inactivation.

Exon deletions preceding exon 51 of the human *DMD* gene, which disrupt the open reading frame (ORF) by juxtaposing out of frame exons, represent the most common type of human DMD mutation. Skipping of exon 51 can, in principle, restore the DMD ORF in 13% of DMD patients with exon deletions.

Duchenne muscular dystrophy has an incidence of 1 in 5000 male infants. Mutations within the dystrophin gene can either be inherited or occur spontaneously during germline transmission.

Sequences

The following tables provide exemplary primer, gRNA and genomic targeting sequences for use in connection with the compositions and methods disclosed herein.

Table 4: Sequence of Primers for DMD iPSCs

PCR/T7E1 and RT-PCR primers					
DMD #	PCR/T7E1	**SEQ ID NO:**	RT-PCR		**SEQ ID NO:**
Del.	F: TTCCCTGGCAAGGTCTGA	2463	F:	CCCAGAAGAGCAAGATAAACTTGAA	2469
	R: ATCCTCAAGGTCACCCACC	2464	R:	CTCTGTTCCAAATCCTGCATTGT	2470
pEx.	F: CACACCTGTTATATTTTTCCGTGAAG	2465	F:	CATAAGCCCAGAAGAGCAAGATAAA	2471
	R: CAAAGGAGAAGCAAAAACACATTCTA	2466	R:	ATAGGAGATAACCACAGCAGCAGAT	2472
Dup.	F: GTAATGTATAACTGTATAACGTGGGGCACTC	2467	E59F:	GGGAAAAATTGAACCTGCAC	2473

	R: GGTGAGTTGTTGCTACAGCTCTTCC	2468	E55R: CATCAGCTCTTTTACTCCCTT	2474
			E53F: GGAGGGTCCCTATACAGTAG	2475

Table 5: Genomic targeting sequences of top 12 exons.

Exon	Applic-ability (30)	gRNA/PAM at acceptor site	SEQ ID NO.	gRNA/PAM at donor site	SEQ ID NO.
51	13.0%	#1: TGCAAAAACCCAAAATATTTTAG	2378		
		#2: AAAATATTTTAGCTCCTACTCAG	2379		
		#3: CAGAGTAACAGTCTGAGTAGGAG*	2380		
45	8.1%	#l: TTGCCTTTTTGGTATCTTACAGG	2381		
		#2: TTTGCCTTTTTGGTATCTTACAG	2382		
		#3: CGCTGCCCAATGCCATCCTGGAG	2383		
53	7.7%	#1: ATTTATTTTTCCTTTATTCTAG	2384	**#4: AAAGAAAATCACAGAAACCAAGG**	**2414**
		#2: TTTCCTTTTATTCTAGTTGAAAG	2385	#5: AAAATCACAGAAACCAAGGTTAG	2415
		#3: TGATTCTGAATTCTTTCAACTAG	2386	**#6: GGTATCTTTGATACTAACCTTGG**	**2416**
44	6.2%	**#1: ATCCATATGCTTTTACCTGCAGG**	2387	**#4: GTAATACAAATGGTATCTTAAGG**	**2417**
		#2: GATCCATATGCTTTTACCTGCAG	2388		
		3: CAGATCTGTCAAATCGCCTGCAG	2389		
46	4.3%	#1: TTATTCTTCTTTCTCCAGGCTAG	2390		
		#2: AATTTTATTCTTCTTTCTCCAGG	2391		
		#3: CAATTTTATTCTTCTTTCTCCAG	2392		
52	4.1%	**#1: TAAGGGATATTTGTTCT**	2393		

		TACAGG			
		#2: CTAAGGGATATTTGTTCT TACAG	2394		
		#3: TGTTCTTACAGGCAACAA TGCAG	2395		
50	4.0%	**#1: TGTATGCTTTTCTGTTA AAGAGG**	2396		
		#2: ATGTGTATGCTTTTCTGTT AAAG	2397		
		#3: GTGTATGCTTTTCTGTTA AAGAG	2398		
43	3.8%	#1: GTTTTAAAATTTTTATATT ACAG	2399	**#4: TATGTGTTACCTACCCTT GTCGG**	**2418**
		#2: TTTTATATTACAGAATAT AAAAG	2400	#5: AAATGTACAAGGACCGAC AAGGG	2419
		#3: ATATTACAGAATATAAAA GATAG	2401	**#6: GTACAAGGACCGACAAGG GTAGG**	**2420**
6	3.0%†	#1: TGAAAATTTATTTCCACA TGTAG	2402	#4: ATGCTCTCATCCATAGTCA TAGG	2421
		#2: GAAAATTTATTTCCACAT GTAGG	2403	#5: TCTCATCCATAGTCATAGG TAAG	2422
		#3: TTACATTTTTGACCTACA TGTGG	2404	#6: CATCCATAGTCATAGGTAA GAAG	2423
7	3.0%†	#1: TGTGTATGTGTATGTGTT TTAGG	2405		
		#2: TATGTGTATGTGTTTTAG GCCAG	2406		
		#3: **CTATTCCAGTCAAATAG GTCTGG**	2407		
8	2.3%	#1: GTGTAGTGTTAATGTGCT TACAG	2408	#4: TGCACTATTCTCAACAGGT AAAG	2424
		#2: GGACTTCTTATCTGGATA GGTGG	2409	**#5: TCAAATGCACTATTCTCAA CAGG**	**2425**
		#3: TAGGTGGTATCAACATCT GTAAG	2410	#6: CTTTACACACTTTACCTGTT GAG	2426

55	2.09%	**#1: TGAACATTTGGTCCTTT GCAGGG**	2411		
		#2: TCTGAACATTTGGTCCTT TGCAG	2412		
		#3: TCTCGCTCACTCACCCT GCAAAG	2413		
		†Dual exon skipping (exons 6 and 7).			

TABLE 6 – Genomic Target Sequences

Targeted gRNA Exon	Guide #	Strand	Genomic Target Sequence*	PAM	SEQ ID NO.
Human-Exon 51	4	1	tctttttcttctttttccttttt	tttt	60
Human-Exon 51	5	1	cttttttcttctttttccttttG	tttt	61
Human-Exon 51	6	1	ttttttcttctttttccttttGC	tttc	62
Human-Exon 51	7	1	tcttctttttttccttttGCAAAA	tttt	63
Human-Exon 51	8	1	cttctttttttccttttGCAAAAA	tttt	64
Human-Exon 51	9	1	ttctttttttccttttGCAAAAAC	tttc	65
Human-Exon 51	10	1	ttcctttttGCAAAAACCCAAAAT	tttt	66
Human-Exon 51	11	1	tcctttttGCAAAAACCCAAAATA	tttt	67
Human-Exon 51	12	1	cctttttGCAAAAACCCAAAATAT	tttt	68
Human-Exon 51	13	1	cttttttGCAAAAACCCAAAATATT	tttc	69
Human-Exon 51	14	1	tGCAAAAACCCAAAATATTTTAGC	tttt	70
Human-Exon 51	15	1	GCAAAAACCCAAAATATTTTAGCT	tttt	71
Human-Exon 51	16	1	CAAAAACCCAAAATATTTTAGCTC	tttG	72
Human-Exon 51	17	1	AGCTCCTACTCAGACTGTTACTCT	TTTT	73
Human-Exon 51	18	1	GCTCCTACTCAGACTGTTACTCTG	TTTA	74
Human-Exon 51	19	-1	CTTAGTAACCACAGGTTGTGTCAC	TTTC	75
Human-Exon 51	20	-1	GAGATGGCAGTTTCCTTAGTAACC	TTTG	76
Human-Exon 51	21	-1	TAGTTTGGAGATGGCAGTTTCCTT	TTTC	77
Human-Exon 51	22	-1	TTCTCATACCTTCTGCTTGATGAT	TTTT	78
Human-Exon 51	23	-1	TCATTTTTTCTCATACCTTCTGCT	TTTA	79
Human-Exon 51	24	-1	ATCATTTTTTCTCATACCTTCTGC	TTTT	80
Human-Exon 51	25	-1	AAGAAAAACTTCTGCCAACTTTTA	TTTA	81
Human-Exon 51	26	-1	AAAGAAAAACTTCTGCCAACTTTT	TTTT	82
Human-Exon 51	27	1	TCTTTAAAATGAAGATTTTCCACC	TTTT	83
Human-Exon 51	28	1	CTTTAAAATGAAGATTTTCCACCA	TTTT	84
Human-Exon 51	29	1	TTTAAAATGAAGATTTTCCACCAA	TTTC	85
Human-Exon 51	30	1	AAATGAAGATTTTCCACCAATCAC	TTTA	86
Human-Exon 51	31	1	CCACCAATCACTTTACTCTCCTAG	TTTT	87
Human-Exon 51	32	1	CACCAATCACTTTACTCTCCTAGA	TTTC	88
Human-Exon 51	33	1	CTCTCCTAGACCATTTCCCACCAG	TTTA	89

Human-Exon 45	1	-1	agaaaagattaaacagtgtgctac	tttg	90
Human-Exon 45	2	-1	tttgagaaaagattaaacagtgtg	TTTa	91
Human-Exon 45	3	-1	atttgagaaaagattaaacagtgt	TTTT	92
Human-Exon 45	4	-1	Tatttgagaaaagattaaacagtg	TTTT	93
Human-Exon 45	5	1	atcttttctcaaatAAAAAGACAT	ttta	94
Human-Exon 45	6	1	ctcaaatAAAAAGACATGGGGCTT	tttt	95
Human-Exon 45	7	1	tcaaatAAAAAGACATGGGGCTTC	tttc	96
Human-Exon 45	8	1	TGTTTTGCCTTTTTGGTATCTTAC	TTTT	97
Human-Exon 45	9	1	GTTTTGCCTTTTTGGTATCTTACA	TTTT	98
Human-Exon 45	10	1	TTTTGCCTTTTTGGTATCTTACAG	TTTG	99
Human-Exon 45	11	1	GCCTTTTTGGTATCTTACAGGAAC	TTTT	100
Human-Exon 45	12	1	CCTTTTTGGTATCTTACAGGAACT	TTTG	101
Human-Exon 45	13	1	TGGTATCTTACAGGAACTCCAGGA	TTTT	102
Human-Exon 45	14	1	GGTATCTTACAGGAACTCCAGGAT	TTTT	103
Human-Exon 45	15	-1	AGGATTGCTGAATTATTTCTTCCC	TTTG	104
Human-Exon 45	16	-1	GAGGATTGCTGAATTATTTCTTCC	TTTT	105
Human-Exon 45	17	-1	TGAGGATTGCTGAATTATTTCTTC	TTTT	106
Human-Exon 45	18	-1	CTGTAGAATACTGGCATCTGTTTT	TTTC	107
Human-Exon 45	19	-1	CCTGTAGAATACTGGCATCTGTTT	TTTT	108
Human-Exon 45	20	-1	TCCTGTAGAATACTGGCATCTGTT	TTTT	109
Human-Exon 45	21	-1	CAGACCTCCTGCCACCGCAGATTC	TTTG	110
Human-Exon 45	22	-1	TGTCTGACAGCTGTTTGCAGACCT	TTTC	111
Human-Exon 45	23	-1	CTGTCTGACAGCTGTTTGCAGACC	TTTT	112
Human-Exon 45	24	-1	TCTGTCTGACAGCTGTTTGCAGAC	TTTT	113
Human-Exon 45	25	-1	TTCTGTCTGACAGCTGTTTGCAGA	TTTT	114
Human-Exon 45	26	-1	ATTCCTATTAGATCTGTCGCCCTA	TTTC	115
Human-Exon 45	27	-1	CATTCCTATTAGATCTGTCGCCCT	TTTT	116
Human-Exon 45	28	1	AGCAGACTTTTTAAGCTTTCTTTA	TTTT	117
Human-Exon 45	29	1	GCAGACTTTTTAAGCTTTCTTTAG	TTTA	118
Human-Exon 45	30	1	TAAGCTTTCTTTAGAAGAATATTT	TTTT	119
Human-Exon 45	31	1	AAGCTTTCTTTAGAAGAATATTTC	TTTT	120
Human-Exon 45	32	1	AGCTTTCTTTAGAAGAATATTTCA	TTTA	121
Human-Exon 45	33	1	TTTAGAAGAATATTTCATGAGAGA	TTTC	122

Human-Exon 45	34	1	GAAGAATATTTCATGAGAGATTAT	TTTA	123
Human-Exon 44	1	1	TCAGTATAACCAAAAAATATACGC	TTTG	124
Human-Exon 44	2	1	acataatccatctatttttcttga	tttt	125
Human-Exon 44	3	1	cataatccatctatttttcttgat	ttta	126
Human-Exon 44	4	1	tcttgatccatatgcttttACCTG	tttt	127
Human-Exon 44	5	1	cttgatccatatgcttttACCTGC	tttt	128
Human-Exon 44	6	1	ttgatccatatgcttttACCTGCA	tttc	129
Human-Exon 44	7	-1	TCAACAGATCTGTCAAATCGCCTG	TTTC	130
Human-Exon 44	8	1	ACCTGCAGGCGATTTGACAGATCT	tttt	131
Human-Exon 44	9	1	CCTGCAGGCGATTTGACAGATCTG	tttA	132
Human-Exon 44	10	1	ACAGATCTGTTGAGAAATGGCGGC	TTTG	133
Human-Exon 44	11	-1	TATCATAATGAAAACGCCGCCATT	TTTA	134
Human-Exon 44	12	1	CATTATGATATAAAGATATTTAAT	TTTT	135
Human-Exon 44	13	-1	TATTTAGCATGTTCCCAATTCTCA	TTTG	136
Human-Exon 44	14	-1	GAAAAAACAAATCAAAGACTTACC	TTTC	137
Human-Exon 44	15	1	ATTTGTTTTTTCGAAATTGTATTT	TTTG	138
Human-Exon 44	16	1	TTTTTTCGAAATTGTATTTATCTT	TTTG	139
Human-Exon 44	17	1	TTCGAAATTGTATTTATCTTCAGC	TTTT	140
Human-Exon 44	18	1	TCGAAATTGTATTTATCTTCAGCA	TTTT	141
Human-Exon 44	19	1	CGAAATTGTATTTATCTTCAGCAC	TTTT	142
Human-Exon 44	20	1	GAAATTGTATTTATCTTCAGCACA	TTTC	143
Human-Exon 44	21	-1	AGAAGTTAAAGAGTCCAGATGTGC	TTTA	144
Human-Exon 44	22	1	TCTTCAGCACATCTGGACTCTTTA	TTTA	145
Human-Exon 44	23	-1	CATCACCCTTCAGAACCTGATCTT	TTTC	146
Human-Exon 44	24	1	ACTTCTTAAAGATCAGGTTCTGAA	TTTA	147
Human-Exon 44	25	1	GACTGTTGTTGTCATCATTATATT	TTTT	148
Human-Exon 44	26	1	ACTGTTGTTGTCATCATTATATTA	TTTG	149
Human-Exon 53	1	-1	AACTAGAATAAAAGGAAAAATAAA	TTTC	150
Human-Exon 53	2	1	CTACTATATATTTATTTTTCCTTT	TTTA	151
Human-Exon 53	3	1	TTTTTCCTTTTATTCTAGTTGAAA	TTTA	152
Human-Exon 53	4	1	TCCTTTTATTCTAGTTGAAAGAAT	TTTT	153
Human-Exon 53	5	1	CCTTTTATTCTAGTTGAAAGAATT	TTTT	154
Human-Exon 53	6	1	CTTTTATTCTAGTTGAAAGAATTC	TTTC	155

Human-Exon 53	7	1	ATTCTAGTTGAAAGAATTCAGAAT	TTTT	156
Human-Exon 53	8	1	TTCTAGTTGAAAGAATTCAGAATC	TTTA	157
Human-Exon 53	9	-1	ATTCAACTGTTGCCTCCGGTTCTG	TTTC	158
Human-Exon 53	10	-1	ACATTTCATTCAACTGTTGCCTCC	TTTA	159
Human-Exon 53	11	-1	CTTTTGGATTGCATCTACTGTATA	TTTT	160
Human-Exon 53	12	-1	TGTGATTTTCTTTTGGATTGCATC	TTTC	161
Human-Exon 53	13	-1	ATACTAACCTTGGTTTCTGTGATT	TTTG	162
Human-Exon 53	14	-1	AAAAGGTATCTTTGATACTAACCT	TTTA	163
Human-Exon 53	15	-1	AAAAAGGTATCTTTGATACTAACC	TTTT	164
Human-Exon 53	16	-1	TTTTAAAAAGGTATCTTTGATACT	TTTA	165
Human-Exon 53	17	-1	ATTTTAAAAAGGTATCTTTGATAC	TTTT	166
Human-Exon 46	1	-1	TTAATGCAAACTGGGACACAAACA	TTTG	167
Human-Exon 46	2	1	TAAATTGCCATGTTTGTGTCCCAG	TTTT	168
Human-Exon 46	3	1	AAATTGCCATGTTTGTGTCCCAGT	TTTT	169
Human-Exon 46	4	1	AATTGCCATGTTTGTGTCCCAGTT	TTTA	170
Human-Exon 46	5	1	TGTCCCAGTTTGCATTAACAAATA	TTTG	171
Human-Exon 46	6	-1	CAACATAGTTCTCAAACTATTTGT	tttC	172
Human-Exon 46	7	-1	CCAACATAGTTCTCAAACTATTTG	tttt	173
Human-Exon 46	8	-1	tCCAACATAGTTCTCAAACTATTT	tttt	174
Human-Exon 46	9	-1	tttCCAACATAGTTCTCAAACTAT	tttt	175
Human-Exon 46	10	-1	ttttCCAACATAGTTCTCAAACTA	tttt	176
Human-Exon 46	11	-1	tttttCCAACATAGTTCTCAAACT	tttt	177
Human-Exon 46	12	1	CATTAACAAATAGTTTGAGAACTA	TTTG	178
Human-Exon 46	13	1	AGAACTATGTTGGaaaaaaaaTA	TTTG	179
Human-Exon 46	14	-1	GTTCTTCTAGCCTGGAGAAAGAAG	TTTT	180
Human-Exon 46	15	1	ATTCTTCTTTCTCCAGGCTAGAAG	TTTT	181
Human-Exon 46	16	1	TTCTTCTTTCTCCAGGCTAGAAGA	TTTA	182
Human-Exon 46	17	1	TCCAGGCTAGAAGAACAAAAGAAT	TTTC	183
Human-Exon 46	18	-1	AAATTCTGACAAGATATTCTTTTG	TTTG	184
Human-Exon 46	19	-1	CTTTTAGTTGCTGCTCTTTTCCAG	TTTT	185
Human-Exon 46	20	-1	AGAAAATAAAATTACCTTGACTTG	TTTG	186
Human-Exon 46	21	-1	TGCAAGCAGGCCCTGGGGGATTTG	TTTA	187
Human-Exon 46	22	1	ATTTTCTCAAATCCCCCAGGGCCT	TTTT	188

Human-Exon 46	23	1	TTTTCTCAAATCCCCCAGGGCCTG	TTTA	189
Human-Exon 46	24	1	CTCAAATCCCCCAGGGCCTGCTTG	TTTT	190
Human-Exon 46	25	1	TCAAATCCCCCAGGGCCTGCTTGC	TTTC	191
Human-Exon 46	26	1	TTAATTCAATCATTGGTTTTCTGC	TTTT	192
Human-Exon 46	27	1	TAATTCAATCATTGGTTTTCTGCC	TTTT	193
Human-Exon 46	28	1	AATTCAATCATTGGTTTTCTGCCC	TTTT	194
Human-Exon 46	29	1	ATTCAATCATTGGTTTTCTGCCCA	TTTA	195
Human-Exon 46	30	-1	GCAAGGAACTATGAATAACCTAAT	TTTA	196
Human-Exon 46	31	1	CTGCCCATTAGGTTATTCATAGTT	TTTT	197
Human-Exon 46	32	1	TGCCCATTAGGTTATTCATAGTTC	TTTC	198
Human-Exon 52	1	-1	TAGAAAACAATTTAACAGGAAATA	TTTA	199
Human-Exon 52	2	1	CTGTTAAATTGTTTTCTATAAACC	TTTC	200
Human-Exon 52	3	-1	GAAATAAAAAAGATGTTACTGTAT	TTTA	201
Human-Exon 52	4	-1	AGAAATAAAAAAGATGTTACTGTA	TTTT	202
Human-Exon 52	5	1	CTATAAACCCTTATACAGTAACAT	TTTT	203
Human-Exon 52	6	1	TATAAACCCTTATACAGTAACATC	TTTC	204
Human-Exon 52	7	1	TTATTTCTAAAAGTGTTTTGGCTG	TTTT	205
Human-Exon 52	8	1	TATTTCTAAAAGTGTTTTGGCTGG	TTTT	206
Human-Exon 52	9	1	ATTTCTAAAAGTGTTTTGGCTGGT	TTTT	207
Human-Exon 52	10	1	TTTCTAAAAGTGTTTTGGCTGGTC	TTTA	208
Human-Exon 52	11	1	TAAAAGTGTTTTGGCTGGTCTCAC	TTTC	209
Human-Exon 52	12	-1	CATAATACAAAGTAAAGTACAATT	TTTA	210
Human-Exon 52	13	-1	ACATAATACAAAGTAAAGTACAAT	TTTT	211
Human-Exon 52	14	1	GGCTGGTCTCACAATTGTACTTTA	TTTT	212
Human-Exon 52	15	1	GCTGGTCTCACAATTGTACTTTAC	TTTG	213
Human-Exon 52	16	1	CTTTGTATTATGTAAAAGGAATAC	TTTA	214
Human-Exon 52	17	1	TATTATGTAAAAGGAATACACAAC	TTTG	215
Human-Exon 52	18	1	TTCTTACAGGCAACAATGCAGGAT	TTTG	216
Human-Exon 52	19	1	GAACAGAGGCGTCCCCAGTTGGAA	TTTG	217
Human-Exon 52	20	-1	GGCAGCGGTAATGAGTTCTTCCAA	TTTG	218
Human-Exon 52	21	-1	TCAAATTTTGGGCAGCGGTAATGA	TTTT	219
Human-Exon 52	22	1	AAAAACAAGACCAGCAATCAAGAG	TTTG	220
Human-Exon 52	23	-1	TGTGTCCCATGCTTGTTAAAAAAC	TTTG	221

Human-Exon 52	24	1	TTAACAAGCATGGGACACACAAAG	TTTT	222
Human-Exon 52	25	1	TAACAAGCATGGGACACACAAAGC	TTTT	223
Human-Exon 52	26	1	AACAAGCATGGGACACACAAAGCA	TTTT	224
Human-Exon 52	27	1	ACAAGCATGGGACACACAAAGCAA	TTTA	225
Human-Exon 52	28	-1	TTGAAACTTGTCATGCATCTTGCT	TTTA	226
Human-Exon 52	29	-1	ATTGAAACTTGTCATGCATCTTGC	TTTT	227
Human-Exon 52	30	-1	TATTGAAACTTGTCATGCATCTTG	TTTT	228
Human-Exon 52	31	1	AATAAAAACTTAAGTTCATATATC	TTTC	229
Human-Exon 50	1	-1	GTGAATATATTATTGGATTTCTAT	TTTG	230
Human-Exon 50	2	-1	AAGATAATTCATGAACATCTTAAT	TTTG	231
Human-Exon 50	3	-1	ACAGAAAAGCATACACATTACTTA	TTTA	232
Human-Exon 50	4	1	CTGTTAAAGAGGAAGTTAGAAGAT	TTTT	233
Human-Exon 50	5	1	TGTTAAAGAGGAAGTTAGAAGATC	TTTC	234
Human-Exon 50	6	-1	CCGCCTTCCACTCAGAGCTCAGAT	TTTA	235
Human-Exon 50	7	-1	CCCTCAGCTCTTGAAGTAAACGGT	TTTG	236
Human-Exon 50	8	1	CTTCAAGAGCTGAGGGCAAAGCAG	TTTA	237
Human-Exon 50	9	-1	AACAAATAGCTAGAGCCAAAGAGA	TTTG	238
Human-Exon 50	10	-1	GAACAAATAGCTAGAGCCAAAGAG	TTTT	239
Human-Exon 50	11	1	GCTCTAGCTATTTGTTCAAAAGTG	TTTG	240
Human-Exon 50	12	1	TTCAAAAGTGCAACTATGAAGTGA	TTTG	241
Human-Exon 50	13	-1	TCTCTCACCCAGTCATCACTTCAT	TTTC	242
Human-Exon 50	14	-1	CTCTCTCACCCAGTCATCACTTCA	TTTT	243
Human-Exon 43	1	1	tatatatatatatatTTTTCTCTT	TTTG	244
Human-Exon 43	2	1	TCTCTTTCTATAGACAGCTAATTC	tTTT	245
Human-Exon 43	3	1	CTCTTTCTATAGACAGCTAATTCA	TTTT	246
Human-Exon 43	4	-1	AAACAGTAAAAAAATGAATTAGCT	TTTA	247
Human-Exon 43	5	1	TCTTTCTATAGACAGCTAATTCAT	TTTC	248
Human-Exon 43	6	-1	AAAACAGTAAAAAAATGAATTAGC	TTTT	249
Human-Exon 43	7	1	TATAGACAGCTAATTCATTTTTTT	TTTC	250
Human-Exon 43	8	-1	TATTCTGTAATATAAAAATTTTAA	TTTA	251
Human-Exon 43	9	-1	ATATTCTGTAATATAAAAATTTTA	TTTT	252
Human-Exon 43	10	1	TTTACTGTTTTAAAATTTTTATAT	TTTT	253
Human-Exon 43	11	1	TTACTGTTTTAAAATTTTTATATT	TTTT	254

Human-Exon 43	12	1	TACTGTTTTAAAATTTTTATATTA	TTTT	255
Human-Exon 43	13	1	ACTGTTTTAAAATTTTTATATTAC	TTTT	256
Human-Exon 43	14	1	CTGTTTTAAAATTTTTATATTACA	TTTA	257
Human-Exon 43	15	1	AAAATTTTTATATTACAGAATATA	TTTT	258
Human-Exon 43	16	1	AAATTTTTATATTACAGAATATAA	TTTA	259
Human-Exon 43	17	-1	TTGTAGACTATCTTTTATATTCTG	TTTG	260
Human-Exon 43	18	1	TATATTACAGAATATAAAAGATAG	TTTT	261
Human-Exon 43	19	1	ATATTACAGAATATAAAAGATAGT	TTTT	262
Human-Exon 43	20	1	TATTACAGAATATAAAAGATAGTC	TTTA	263
Human-Exon 43	21	-1	CAATGCTGCTGTCTTCTTGCTATG	TTTG	264
Human-Exon 43	22	1	CAATGGGAAAAAGTTAACAAAATG	TTTC	265
Human-Exon 43	23	-1	TGCAAGTATCAAGAAAAATATATG	TTTC	266
Human-Exon 43	24	1	TCTTGATACTTGCAGAAATGATTT	TTTT	267
Human-Exon 43	25	1	CTTGATACTTGCAGAAATGATTTG	TTTT	268
Human-Exon 43	26	1	TTGATACTTGCAGAAATGATTTGT	TTTC	269
Human-Exon 43	27	1	TTTTCAGGGAACTGTAGAATTTAT	TTTG	270
Human-Exon 43	28	-1	CATGGAGGGTACTGAAATAAATTC	TTTC	271
Human-Exon 43	29	-1	CCATGGAGGGTACTGAAATAAATT	TTTT	272
Human-Exon 43	30	1	CAGGGAACTGTAGAATTTATTTCA	TTTT	273
Human-Exon 43	31	-1	TCCATGGAGGGTACTGAAATAAAT	TTTT	274
Human-Exon 43	32	1	AGGGAACTGTAGAATTTATTTCAG	TTTC	275
Human-Exon 43	33	-1	TTCCATGGAGGGTACTGAAATAAA	TTTT	276
Human-Exon 43	34	-1	CCTGTCTTTTTTCCATGGAGGGTA	TTTC	277
Human-Exon 43	35	-1	CCCTGTCTTTTTTCCATGGAGGGT	TTTT	278
Human-Exon 43	36	-1	TCCCTGTCTTTTTTCCATGGAGGG	TTTT	279
Human-Exon 43	37	1	TTTCAGTACCCTCCATGGAAAAAA	TTTA	280
Human-Exon 43	38	1	AGTACCCTCCATGGAAAAAAGACA	TTTC	281
Human-Exon 6	1	1	AGTTTGCATGGTTCTTGCTCAAGG	TTTA	282
Human-Exon 6	2	-1	ATAAGAAAATGCATTCCTTGAGCA	TTTC	283
Human-Exon 6	3	-1	CATAAGAAAATGCATTCCTTGAGC	TTTT	284
Human-Exon 6	4	1	CATGGTTCTTGCTCAAGGAATGCA	TTTG	285
Human-Exon 6	5	-1	ACCTACATGTGGAAATAAATTTTC	TTTG	286
Human-Exon 6	6	-1	GACCTACATGTGGAAATAAATTTT	TTTT	287

Human-Exon 6	7	-1	TGACCTACATGTGGAAATAAATTT	TTTT	288
Human-Exon 6	8	1	CTTATGAAAATTTATTTCCACATG	TTTT	289
Human-Exon 6	9	1	TTATGAAAATTTATTTCCACATGT	TTTC	290
Human-Exon 6	10	-1	ATTACATTTTTGACCTACATGTGG	TTTC	291
Human-Exon 6	11	-1	CATTACATTTTTGACCTACATGTG	TTTT	292
Human-Exon 6	12	-1	TCATTACATTTTTGACCTACATGT	TTTT	293
Human-Exon 6	13	1	TTTCCACATGTAGGTCAAAAATGT	TTTA	294
Human-Exon 6	14	1	CACATGTAGGTCAAAAATGTAATG	TTTC	295
Human-Exon 6	15	-1	TTGCAATCCAGCCATGATATTTTT	TTTG	296
Human-Exon 6	16	-1	ACTGTTGGTTTGTTGCAATCCAGC	TTTC	297
Human-Exon 6	17	-1	CACTGTTGGTTTGTTGCAATCCAG	TTTT	298
Human-Exon 6	18	1	AATGCTCTCATCCATAGTCATAGG	TTTG	299
Human-Exon 6	19	-1	ATGTCTCAGTAATCTTCTTACCTA	TTTA	300
Human-Exon 6	20	-1	CAAGTTATTTAATGTCTCAGTAAT	TTTA	301
Human-Exon 6	21	-1	ACAAGTTATTTAATGTCTCAGTAA	TTTT	302
Human-Exon 6	22	1	GACTCTGATGACATATTTTTCCCC	TTTA	303
Human-Exon 6	23	1	TCCCCAGTATGGTTCCAGATCATG	TTTT	304
Human-Exon 6	24	1	CCCCAGTATGGTTCCAGATCATGT	TTTT	305
Human-Exon 6	25	1	CCCAGTATGGTTCCAGATCATGTC	TTTC	306
Human-Exon 7	1	1	TATTTGTCTTtgtgtatgtgtgta	TTTA	307
Human-Exon 7	2	1	TCTTtgtgtatgtgtgtatgtgta	TTTG	308
Human-Exon 7	3	1	tgtatgtgtgtatgtgtatgtgtt	TTtg	309
Human-Exon 7	4	1	AGGCCAGACCTATTTGACTGGAAT	ttTT	310
Human-Exon 7	5	1	GGCCAGACCTATTTGACTGGAATA	tTTA	311
Human-Exon 7	6	1	ACTGGAATAGTGTGGTTTGCCAGC	TTTG	312
Human-Exon 7	7	1	CCAGCAGTCAGCCACACAACGACT	TTTG	313
Human-Exon 7	8	-1	TCTATGCCTAATTGATATCTGGCG	TTTC	314
Human-Exon 7	9	-1	CCAACCTTCAGGATCGAGTAGTTT	TTTA	315
Human-Exon 7	10	1	TGGACTACCACTGCTTTTAGTATG	TTTC	316
Human-Exon 7	11	1	AGTATGGTAGAGTTTAATGTTTTC	TTTT	317
Human-Exon 7	12	1	GTATGGTAGAGTTTAATGTTTTCA	TTTA	318
Human-Exon 8	1	-1	AGACTCTAAAAGGATAATGAACAA	TTTG	319
Human-Exon 8	2	1	ACTTTGATTTGTTCATTATCCTTT	TTTA	320

Human-Exon 8	3	-1	TATATTTGAGACTCTAAAAGGATA	TTTC	321
Human-Exon 8	4	1	ATTTGTTCATTATCCTTTTAGAGT	TTTG	322
Human-Exon 8	5	-1	GTTTCTATATTTGAGACTCTAAAA	TTTG	323
Human-Exon 8	6	-1	GGTTTCTATATTTGAGACTCTAAA	TTTT	324
Human-Exon 8	7	-1	TGGTTTCTATATTTGAGACTCTAA	TTTT	325
Human-Exon 8	8	1	TTCATTATCCTTTTAGAGTCTCAA	TTTG	326
Human-Exon 8	9	1	AGAGTCTCAAATATAGAAACCAAA	TTTT	327
Human-Exon 8	10	1	GAGTCTCAAATATAGAAACCAAAA	TTTA	328
Human-Exon 8	11	-1	CACTTCCTGGATGGCTTCAATGCT	TTTC	329
Human-Exon 8	12	1	GCCTCAACAAGTGAGCATTGAAGC	TTTT	330
Human-Exon 8	13	1	CCTCAACAAGTGAGCATTGAAGCC	TTTG	331
Human-Exon 8	14	-1	GGTGGCCTTGGCAACATTTCCACT	TTTA	332
Human-Exon 8	15	-1	GTCACTTTAGGTGGCCTTGGCAAC	TTTA	333
Human-Exon 8	16	-1	ATGATGTAACTGAAAATGTTCTTC	TTTG	334
Human-Exon 8	17	-1	CCTGTTGAGAATAGTGCATTTGAT	TTTA	335
Human-Exon 8	18	1	CAGTTACATCATCAAATGCACTAT	TTTT	336
Human-Exon 8	19	1	AGTTACATCATCAAATGCACTATT	TTTC	337
Human-Exon 8	20	-1	CACACTTTACCTGTTGAGAATAGT	TTTA	338
Human-Exon 8	21	1	CTGTTTTATATGCATTTTTAGGTA	TTTT	339
Human-Exon 8	22	1	TGTTTTATATGCATTTTTAGGTAT	TTTC	340
Human-Exon 8	23	1	ATATGCATTTTTAGGTATTACGTG	TTTT	341
Human-Exon 8	24	1	TATGCATTTTTAGGTATTACGTGC	TTTA	342
Human-Exon 8	25	1	TAGGTATTACGTGCACatatatat	TTTT	343
Human-Exon 8	26	1	AGGTATTACGTGCACatatatata	TTTT	344
Human-Exon 8	27	1	GGTATTACGTGCACatatatatat	TTTA	345
Human-Exon 55	1	-1	AGCAACAACTATAATATTGTGCAG	TTTA	346
Human-Exon 55	2	1	GTTCCTCCATCTTTCTCTTTTTAT	TTTA	347
Human-Exon 55	3	1	TCTTTTTATGGAGTTCACTAGGTG	TTTC	348
Human-Exon 55	4	1	TATGGAGTTCACTAGGTGCACCAT	TTTT	349
Human-Exon 55	5	1	ATGGAGTTCACTAGGTGCACCATT	TTTT	350
Human-Exon 55	6	1	TGGAGTTCACTAGGTGCACCATTC	TTTA	351
Human-Exon 55	7	1	ATAATTGCATCTGAACATTTGGTC	TTTA	352
Human-Exon 55	8	1	GTCCTTTGCAGGGTGAGTGAGCGA	TTTG	353

Human-Exon 55	9	-1	TTCCAAAGCAGCCTCTCGCTCACT	TTTC	354
Human-Exon 55	10	1	CAGGGTGAGTGAGCGAGAGGCTGC	TTTG	355
Human-Exon 55	11	1	GAAGAAACTCATAGATTACTGCAA	TTTG	356
Human-Exon 55	12	-1	CAGGTCCAGGGGGAACTGTTGCAG	TTTC	357
Human-Exon 55	13	-1	CCAGGTCCAGGGGGAACTGTTGCA	TTTT	358
Human-Exon 55	14	-1	AGCTTCTGTAAGCCAGGCAAGAAA	TTTC	359
Human-Exon 55	15	1	TTGCCTGGCTTACAGAAGCTGAAA	TTTC	360
Human-Exon 55	16	-1	CTTACGGGTAGCATCCTGTAGGAC	TTTC	361
Human-Exon 55	17	-1	CTCCCTTGGAGTCTTCTAGGAGCC	TTTA	362
Human-Exon 55	18	-1	ACTCCCTTGGAGTCTTCTAGGAGC	TTTT	363
Human-Exon 55	19	-1	ATCAGCTCTTTTACTCCCTTGGAG	TTTC	364
Human-Exon 55	20	1	CGCTTTAGCACTCTTGTGGATCCA	TTTC	365
Human-Exon 55	21	1	GCACTCTTGTGGATCCAATTGAAC	TTTA	366
Human-Exon 55	22	-1	TCCCTGGCTTGTCAGTTACAAGTA	TTTG	367
Human-Exon 55	23	-1	GTCCCTGGCTTGTCAGTTACAAGT	TTTT	368
Human-Exon 55	24	-1	TTTTGTCCCTGGCTTGTCAGTTAC	TTTG	369
Human-Exon 55	25	-1	GTTTTGTCCCTGGCTTGTCAGTTA	TTTT	370
Human-Exon 55	26	1	TACTTGTAACTGACAAGCCAGGGA	TTTG	371
Human-G1-exon51		1	gCTCCTACTCAGACTGTTACTCTG	TTTA	372
Human-G2-exon51		1	taccatgtattgctaaacaaagta	TTTC	373
Human-G3-exon51		-1	attgaagagtaacaatttgagcca	TTTA	374
mouse-Exon23-G1		1	aggctctgcaaagttctTTGAAAG	TTTG	375
mouse-Exon23-G2		1	AAAGAGCAACAAAATGGCttcaac	TTTG	376
mouse-Exon23-G3		1	AAAGAGCAATAAAATGGCttcaac	TTTG	377
mouse-Exon23-G4		-1	AAAGAACTTTGCAGAGCctcaaaa	TTTC	378
mouse-Exon23-G5		-1	ctgaatatctatgcattaataact	TTTA	379
mouse-Exon23-G6		-1	tattatattacagggcatattata	TTTC	380
mouse-Exon23-G7		1	Aggtaagccgaggtttggccttta	TTTC	381

mouse-Exon23-G8		1	cccagagtccttcaaagatattga	TTTA	382

* In this table, upper case letters represent nucleotides that align to the exon sequence of the gene. Lower case letters represent nucleotides that align to the intron sequence of the gene.

TABLE 7 – gRNA sequences

Targeted gRNA Exon	Guide #	Strand	gRNA sequence*	PAM	SEQ ID NO.
Human-Exon 51	4	1	aaaaaggaaaaaagaagaaaaaga	tttt	383
Human-Exon 51	5	1	Caaaaaggaaaaaagaagaaaaag	tttt	384
Human-Exon 51	6	1	GCaaaaaggaaaaaagaagaaaaa	tttc	385
Human-Exon 51	7	1	UUUUGCaaaaaggaaaaaagaaga	tttt	386
Human-Exon 51	8	1	UUUUUGCaaaaaggaaaaaagaag	tttt	387
Human-Exon 51	9	1	GUUUUUGCaaaaaggaaaaaagaa	tttc	388
Human-Exon 51	10	1	AUUUUGGGUUUUUGCaaaaaggaa	tttt	389
Human-Exon 51	11	1	UAUUUUGGGUUUUUGCaaaaagga	tttt	390
Human-Exon 51	12	1	AUAUUUUGGGUUUUUGCaaaaagg	tttt	391
Human-Exon 51	13	1	AAUAUUUUGGGUUUUUGCaaaaag	tttc	392
Human-Exon 51	14	1	GCUAAAAUAUUUUGGGUUUUUGCa	tttt	393
Human-Exon 51	15	1	AGCUAAAAUAUUUUGGGUUUUUGC	tttt	394
Human-Exon 51	16	1	GAGCUAAAAUAUUUUGGGUUUUUG	tttG	395
Human-Exon 51	17	1	AGAGUAACAGUCUGAGUAGGAGCU	TTTT	396
Human-Exon 51	18	1	CAGAGUAACAGUCUGAGUAGGAGC	TTTA	397
Human-Exon 51	19	-1	GUGACACAACCUGUGGUUACUAAG	TTTC	398
Human-Exon 51	20	-1	GGUUACUAAGGAAACUGCCAUCU	TTTG	399
Human-Exon 51	21	-1	AAGGAAACUGCCAUCUCCAAACUA	TTTC	400
Human-Exon 51	22	-1	AUCAUCAAGCAGAAGGUAUGAGAA	TTTT	401
Human-Exon 51	23	-1	AGCAGAAGGUAUGAGAAAAAAUGA	TTTA	402
Human-Exon 51	24	-1	GCAGAAGGUAUGAGAAAAAAUGAU	TTTT	403
Human-Exon 51	25	-1	UAAAAGUUGGCAGAAGUUUUUCUU	TTTA	404
Human-Exon 51	26	-1	AAAAGUUGGCAGAAGUUUUUCUUU	TTTT	405

Human-Exon 51	27	1	GGUGGAAAAUCUUCAUUUUAAAGA	TTTT	406
Human-Exon 51	28	1	UGGUGGAAAAUCUUCAUUUUAAAG	TTTT	407
Human-Exon 51	29	1	UUGGUGGAAAAUCUUCAUUUUAAA	TTTC	408
Human-Exon 51	30	1	GUGAUUGGUGGAAAAUCUUCAUUU	TTTA	409
Human-Exon 51	31	1	CUAGGAGAGUAAAGUGAUUGGUGG	TTTT	410
Human-Exon 51	32	1	UCUAGGAGAGUAAAGUGAUUGGUG	TTTC	411
Human-Exon 51	33	1	CUGGUGGGAAAUGGUCUAGGAGA	TTTA	412
Human-Exon 45	1	-1	guagcacacuguuuaaaucuuuucu	tttg	413
Human-Exon 45	2	-1	cacacuguuuaaaucuuuucucaaa	TTTa	414
Human-Exon 45	3	-1	acacuguuuaaaucuuuucucaaau	TTTT	415
Human-Exon 45	4	-1	cacuguuuaaaucuuuucucaaauA	TTTT	416
Human-Exon 45	5	1	AUGUCUUUUUauuugagaaaagau	ttta	417
Human-Exon 45	6	1	AAGCCCCAUGUCUUUUUauuugag	tttt	418
Human-Exon 45	7	1	GAAGCCCCAUGUCUUUUUauuuga	tttc	419
Human-Exon 45	8	1	GUAAGAUACCAAAAAGGCAAAACA	TTTT	420
Human-Exon 45	9	1	UGUAAGAUACCAAAAAGGCAAAAC	TTTT	421
Human-Exon 45	10	1	CUGUAAGAUACCAAAAAGGCAAAA	TTTG	422
Human-Exon 45	11	1	GUUCCUGUAAGAUACCAAAAAGGC	TTTT	423
Human-Exon 45	12	1	AGUUCCUGUAAGAUACCAAAAAGG	TTTG	424
Human-Exon 45	13	1	UCCUGGAGUUCCUGUAAGAUACCA	TTTT	425
Human-Exon 45	14	1	AUCCUGGAGUUCCUGUAAGAUACC	TTTT	426
Human-Exon 45	15	-1	GGGAAGAAAUAAUUCAGCAAUCCU	TTTG	427
Human-Exon 45	16	-1	GGAAGAAAUAAUUCAGCAAUCCUC	TTTT	428
Human-Exon 45	17	-1	GAAGAAAUAAUUCAGCAAUCCUCA	TTTT	429
Human-Exon 45	18	-1	AAAACAGAUGCCAGUAUUCUACAG	TTTC	430
Human-Exon 45	19	-1	AAACAGAUGCCAGUAUUCUACAGG	TTTT	431
Human-Exon 45	20	-1	AACAGAUGCCAGUAUUCUACAGGA	TTTT	432
Human-Exon 45	21	-1	GAAUCUGCGGUGGCAGGAGGUCUG	TTTG	433
Human-Exon 45	22	-1	AGGUCUGCAAACAGCUGUCAGACA	TTTC	434
Human-Exon 45	23	-1	GGUCUGCAAACAGCUGUCAGACAG	TTTT	435
Human-Exon 45	24	-1	GUCUGCAAACAGCUGUCAGACAGA	TTTT	436
Human-Exon 45	25	-1	UCUGCAAACAGCUGUCAGACAGAA	TTTT	437
Human-Exon 45	26	-1	UAGGGCGACAGAUCUAAUAGGAAU	TTTC	438

Human-Exon 45	27	-1	AGGGCGACAGAUCUAAUAGGAAUG	TTTT	439
Human-Exon 45	28	1	UAAAGAAAGCUUAAAAAGUCUGCU	TTTT	440
Human-Exon 45	29	1	CUAAAGAAAGCUUAAAAAGUCUGC	TTTA	441
Human-Exon 45	30	1	AAAUAUUCUUCUAAAGAAAGCUUA	TTTT	442
Human-Exon 45	31	1	GAAAUAUUCUUCUAAAGAAAGCUU	TTTT	443
Human-Exon 45	32	1	UGAAAUAUUCUUCUAAAGAAAGCU	TTTA	444
Human-Exon 45	33	1	UCUCUCAUGAAAUAUUCUUCUAAA	TTTC	445
Human-Exon 45	34	1	AUAAUCUCUCAUGAAAUAUUCUUC	TTTA	446
Human-Exon 44	1	1	GCGUAUAUUUUUUGGUUAUACUGA	TTTG	447
Human-Exon 44	2	1	ucaagaaaaauagauggauuaugu	tttt	448
Human-Exon 44	3	1	aucaagaaaaauagauggauuaug	ttta	449
Human-Exon 44	4	1	CAGGUaaaagcauauggaucaaga	tttt	450
Human-Exon 44	5	1	GCAGGUaaaagcauauggaucaag	tttt	451
Human-Exon 44	6	1	UGCAGGUaaaagcauauggaucaa	tttc	452
Human-Exon 44	7	-1	CAGGCGAUUUGACAGAUCUGUUGA	TTTC	453
Human-Exon 44	8	1	AGAUCUGUCAAAUCGCCUGCAGGU	tttt	454
Human-Exon 44	9	1	CAGAUCUGUCAAAUCGCCUGCAGG	tttA	455
Human-Exon 44	10	1	GCCGCCAUUUCUCAACAGAUCUGU	TTTG	456
Human-Exon 44	11	-1	AAUGGCGGCGUUUUCAUUAUGAUA	TTTA	457
Human-Exon 44	12	1	AUUAAAUAUCUUUAUAUCAUAAUG	TTTT	458
Human-Exon 44	13	-1	UGAGAAUUGGGAACAUGCUAAAUA	TTTG	459
Human-Exon 44	14	-1	GGUAAGUCUUUGAUUUGUUUUUUC	TTTC	460
Human-Exon 44	15	1	AAAUACAAUUUCGAAAAAACAAAU	TTTG	461
Human-Exon 44	16	1	AAGAUAAAUACAAUUUCGAAAAAA	TTTG	462
Human-Exon 44	17	1	GCUGAAGAUAAAUACAAUUUCGAA	TTTT	463
Human-Exon 44	18	1	UGCUGAAGAUAAAUACAAUUUCGA	TTTT	464
Human-Exon 44	19	1	GUGCUGAAGAUAAAUACAAUUUCG	TTTT	465
Human-Exon 44	20	1	UGUGCUGAAGAUAAAUACAAUUUC	TTTC	466
Human-Exon 44	21	-1	GCACAUCUGGACUCUUUAACUUCU	TTTA	467
Human-Exon 44	22	1	UAAAGAGUCCAGAUGUGCUGAAGA	TTTA	468
Human-Exon 44	23	-1	AAGAUCAGGUUCUGAAGGGUGAUG	TTTC	469
Human-Exon 44	24	1	UUCAGAACCUGAUCUUUAAGAAGU	TTTA	470
Human-Exon 44	25	1	AAUAUAAUGAUGACAACAACAGUC	TTTT	471

Human-Exon 44	26	1	UAAUAUAAUGAUGACAACAACAGU	TTTG	472
Human-Exon 53	1	-1	UUUAUUUUUCCUUUUAUUCUAGUU	TTTC	473
Human-Exon 53	2	1	AAAGGAAAAAUAAAUAUAUAGUAG	TTTA	474
Human-Exon 53	3	1	UUUCAACUAGAAUAAAAGGAAAAA	TTTA	475
Human-Exon 53	4	1	AUUCUUUCAACUAGAAUAAAAGGA	TTTT	476
Human-Exon 53	5	1	AAUUCUUUCAACUAGAAUAAAAGG	TTTT	477
Human-Exon 53	6	1	GAAUUCUUUCAACUAGAAUAAAAG	TTTC	478
Human-Exon 53	7	1	AUUCUGAAUUCUUUCAACUAGAAU	TTTT	479
Human-Exon 53	8	1	GAUUCUGAAUUCUUUCAACUAGAA	TTTA	480
Human-Exon 53	9	-1	CAGAACCGGAGGCAACAGUUGAAU	TTTC	481
Human-Exon 53	10	-1	GGAGGCAACAGUUGAAUGAAAUGU	TTTA	482
Human-Exon 53	11	-1	UAUACAGUAGAUGCAAUCCAAAAG	TTTT	483
Human-Exon 53	12	-1	GAUGCAAUCCAAAAGAAAAUCACA	TTTC	484
Human-Exon 53	13	-1	AAUCACAGAAACCAAGGUUAGUAU	TTTG	485
Human-Exon 53	14	-1	AGGUUAGUAUCAAAGAUACCUUU	TTTA	486
Human-Exon 53	15	-1	GGUUAGUAUCAAAGAUACCUUUUU	TTTT	487
Human-Exon 53	16	-1	AGUAUCAAAGAUACCUUUUUAAAA	TTTA	488
Human-Exon 53	17	-1	GUAUCAAAGAUACCUUUUUAAAAU	TTTT	489
Human-Exon 46	1	-1	UGUUUGUGUCCCAGUUUGCAUUAA	TTTG	490
Human-Exon 46	2	1	CUGGGACACAAACAUGGCAAUUUA	TTTT	491
Human-Exon 46	3	1	ACUGGGACACAAACAUGGCAAUUU	TTTT	492
Human-Exon 46	4	1	AACUGGGACACAAACAUGGCAAUU	TTTA	493
Human-Exon 46	5	1	UAUUUGUUAAUGCAAACUGGGACA	TTTG	494
Human-Exon 46	6	-1	ACAAAUAGUUUGAGAACUAUGUUG	tttC	495
Human-Exon 46	7	-1	CAAAUAGUUUGAGAACUAUGUUGG	tttt	496
Human-Exon 46	8	-1	AAAUAGUUUGAGAACUAUGUUGGa	tttt	497
Human-Exon 46	9	-1	AUAGUUUGAGAACUAUGUUGGaaa	tttt	498
Human-Exon 46	10	-1	UAGUUUGAGAACUAUGUUGGaaaa	tttt	499
Human-Exon 46	11	-1	AGUUUGAGAACUAUGUUGGaaaaa	tttt	500
Human-Exon 46	12	1	UAGUUCUCAAACUAUUUGUUAAUG	TTTG	501
Human-Exon 46	13	1	UAuuuuuuuuuCCAACAUAGUUCU	TTTG	502
Human-Exon 46	14	-1	CUUCUUUCUCCAGGCUAGAAGAAC	TTTT	503
Human-Exon 46	15	1	CUUCUAGCCUGGAGAAAGAAGAAU	TTTT	504

Human-Exon 46	16	1	UCUUCUAGCCUGGAGAAAGAAGAA	TTTA	505
Human-Exon 46	17	1	AUUCUUUUGUUCUUCUAGCCUGGA	TTTC	506
Human-Exon 46	18	-1	CAAAAGAAUAUCUUGUCAGAAUUU	TTTG	507
Human-Exon 46	19	-1	CUGGAAAAGAGCAGCAACUAAAAG	TTTT	508
Human-Exon 46	20	-1	CAAGUCAAGGUAAUUUUAUUUUCU	TTTG	509
Human-Exon 46	21	-1	CAAAUCCCCCAGGGCCUGCUUGCA	TTTA	510
Human-Exon 46	22	1	AGGCCCUGGGGGAUUUGAGAAAAU	TTTT	511
Human-Exon 46	23	1	CAGGCCCUGGGGGAUUUGAGAAAA	TTTA	512
Human-Exon 46	24	1	CAAGCAGGCCCUGGGGGAUUUGAG	TTTT	513
Human-Exon 46	25	1	GCAAGCAGGCCCUGGGGGAUUUGA	TTTC	514
Human-Exon 46	26	1	GCAGAAAACCAAUGAUUGAAUUAA	TTTT	515
Human-Exon 46	27	1	GGCAGAAAACCAAUGAUUGAAUUA	TTTT	516
Human-Exon 46	28	1	GGGCAGAAAACCAAUGAUUGAAUU	TTTT	517
Human-Exon 46	29	1	UGGGCAGAAAACCAAUGAUUGAAU	TTTA	518
Human-Exon 46	30	-1	AUUAGGUUAUUCAUAGUUCCUUGC	TTTA	519
Human-Exon 46	31	1	AACUAUGAAUAACCUAAUGGGCAG	TTTT	520
Human-Exon 46	32	1	GAACUAUGAAUAACCUAAUGGGCA	TTTC	521
Human-Exon 52	1	-1	UAUUUCCUGUUAAAUUGUUUUCUA	TTTA	522
Human-Exon 52	2	1	GGUUUAUAGAAAACAAUUUAACAG	TTTC	523
Human-Exon 52	3	-1	AUACAGUAACAUCUUUUUUAUUUC	TTTA	524
Human-Exon 52	4	-1	UACAGUAACAUCUUUUUUAUUUCU	TTTT	525
Human-Exon 52	5	1	AUGUUACUGUAUAAGGGUUUAUAG	TTTT	526
Human-Exon 52	6	1	GAUGUUACUGUAUAAGGGUUUAUA	TTTC	527
Human-Exon 52	7	1	CAGCCAAAACACUUUUAGAAAUAA	TTTT	528
Human-Exon 52	8	1	CCAGCCAAAACACUUUUAGAAAUA	TTTT	529
Human-Exon 52	9	1	ACCAGCCAAAACACUUUUAGAAAU	TTTT	530
Human-Exon 52	10	1	GACCAGCCAAAACACUUUUAGAAA	TTTA	531
Human-Exon 52	11	1	GUGAGACCAGCCAAAACACUUUUA	TTTC	532
Human-Exon 52	12	-1	AAUUGUACUUUACUUUGUAUUAUG	TTTA	533
Human-Exon 52	13	-1	AUUGUACUUUACUUUGUAUUAUGU	TTTT	534
Human-Exon 52	14	1	UAAAGUACAAUUGUGAGACCAGCC	TTTT	535
Human-Exon 52	15	1	GUAAAGUACAAUUGUGAGACCAGC	TTTG	536
Human-Exon 52	16	1	GUAUUCCUUUUACAUAAUACAAAG	TTTA	537

Human-Exon 52	17	1	GUUGUGUAUUCCUUUUACAUAAUA	TTTG	538
Human-Exon 52	18	1	AUCCUGCAUUGUUGCCUGUAAGAA	TTTG	539
Human-Exon 52	19	1	UUCCAACUGGGGACGCCUCUGUUC	TTTG	540
Human-Exon 52	20	-1	UUGGAAGAACUCAUUACCGCUGCC	TTTG	541
Human-Exon 52	21	-1	UCAUUACCGCUGCCCAAAAUUUGA	TTTT	542
Human-Exon 52	22	1	CUCUUGAUUGCUGGUCUUGUUUUU	TTTG	543
Human-Exon 52	23	-1	GUUUUUUAACAAGCAUGGGACACA	TTTG	544
Human-Exon 52	24	1	CUUUGUGUGUCCCAUGCUUGUUAA	TTTT	545
Human-Exon 52	25	1	GCUUUGUGUGUCCCAUGCUUGUUA	TTTT	546
Human-Exon 52	26	1	UGCUUUGUGUGUCCCAUGCUUGUU	TTTT	547
Human-Exon 52	27	1	UUGCUUUGUGUGUCCCAUGCUUGU	TTTA	548
Human-Exon 52	28	-1	AGCAAGAUGCAUGACAAGUUUCAA	TTTA	549
Human-Exon 52	29	-1	GCAAGAUGCAUGACAAGUUUCAAU	TTTT	550
Human-Exon 52	30	-1	CAAGAUGCAUGACAAGUUUCAAUA	TTTT	551
Human-Exon 52	31	1	GAUAUAUGAACUUAAGUUUUUAUU	TTTC	552
Human-Exon 50	1	-1	AUAGAAAUCCAAUAAUAUAUUCAC	TTTG	553
Human-Exon 50	2	-1	AUUAAGAUGUUCAUGAAUUAUCUU	TTTG	554
Human-Exon 50	3	-1	UAAGUAAUGUGUAUGCUUUUCUGU	TTTA	555
Human-Exon 50	4	1	AUCUUCUAACUUCCUCUUUAACAG	TTTT	556
Human-Exon 50	5	1	GAUCUUCUAACUUCCUCUUUAACA	TTTC	557
Human-Exon 50	6	-1	AUCUGAGCUCUGAGUGGAAGGCGG	TTTA	558
Human-Exon 50	7	-1	ACCGUUUACUUCAAGAGCUGAGGG	TTTG	559
Human-Exon 50	8	1	CUGCUUUGCCCUCAGCUCUUGAAG	TTTA	560
Human-Exon 50	9	-1	UCUCUUUGGCUCUAGCUAUUUGUU	TTTG	561
Human-Exon 50	10	-1	CUCUUUGGCUCUAGCUAUUUGUUC	TTTT	562
Human-Exon 50	11	1	CACUUUUGAACAAAUAGCUAGAGC	TTTG	563
Human-Exon 50	12	1	UCACUUCAUAGUUGCACUUUUGAA	TTTG	564
Human-Exon 50	13	-1	AUGAAGUGAUGACUGGGUGAGAGA	TTTC	565
Human-Exon 50	14	-1	UGAAGUGAUGACUGGGUGAGAGAG	TTTT	566
Human-Exon 43	1	1	AAGAGAAAAauauauauauauaua	TTTG	567
Human-Exon 43	2	1	GAAUUAGCUGUCUAUAGAAAGAGA	tTTT	568
Human-Exon 43	3	1	UGAAUUAGCUGUCUAUAGAAAGAG	TTTT	569
Human-Exon 43	4	-1	AGCUAAUUCAUUUUUUUACUGUUU	TTTA	570

Human-Exon 43	5	1	AUGAAUUAGCUGUCUAUAGAAAGA	TTTC	571
Human-Exon 43	6	-1	GCUAAUUCAUUUUUUUACUGUUUU	TTTT	572
Human-Exon 43	7	1	AAAAAAAUGAAUUAGCUGUCUAUA	TTTC	573
Human-Exon 43	8	-1	UUAAAAUUUUUAUAUUACAGAAUA	TTTA	574
Human-Exon 43	9	-1	UAAAAUUUUUAUAUUACAGAAUAU	TTTT	575
Human-Exon 43	10	1	AUAUAAAAAUUUUAAAACAGUAAA	TTTT	576
Human-Exon 43	11	1	AAUAUAAAAAUUUUAAAACAGUAA	TTTT	577
Human-Exon 43	12	1	UAAUAUAAAAAUUUUAAAACAGUA	TTTT	578
Human-Exon 43	13	1	GUAAUAUAAAAAUUUUAAAACAGU	TTTT	579
Human-Exon 43	14	1	UGUAAUAUAAAAAUUUUAAAACAG	TTTA	580
Human-Exon 43	15	1	UAUAUUCUGUAAUAUAAAAAUUUU	TTTT	581
Human-Exon 43	16	1	UUAUAUUCUGUAAUAUAAAAAUUU	TTTA	582
Human-Exon 43	17	-1	CAGAAUAUAAAAGAUAGUCUACAA	TTTG	583
Human-Exon 43	18	1	CUAUCUUUUAUAUUCUGUAAUAUA	TTTT	584
Human-Exon 43	19	1	ACUAUCUUUUAUAUUCUGUAAUAU	TTTT	585
Human-Exon 43	20	1	GACUAUCUUUUAUAUUCUGUAAUA	TTTA	586
Human-Exon 43	21	-1	CAUAGCAAGAAGACAGCAGCAUUG	TTTG	587
Human-Exon 43	22	1	CAUUUUGUUAACUUUUUCCCAUUG	TTTC	588
Human-Exon 43	23	-1	CAUAUAUUUUUCUUGAUACUUGCA	TTTC	589
Human-Exon 43	24	1	AAAUCAUUUCUGCAAGUAUCAAGA	TTTT	590
Human-Exon 43	25	1	CAAAUCAUUUCUGCAAGUAUCAAG	TTTT	591
Human-Exon 43	26	1	ACAAAUCAUUUCUGCAAGUAUCAA	TTTC	592
Human-Exon 43	27	1	AUAAAUUCUACAGUUCCCUGAAAA	TTTG	593
Human-Exon 43	28	-1	GAAUUUAUUUCAGUACCCUCCAUG	TTTC	594
Human-Exon 43	29	-1	AAUUUAUUUCAGUACCCUCCAUGG	TTTT	595
Human-Exon 43	30	1	UGAAAUAAAUUCUACAGUUCCCUG	TTTT	596
Human-Exon 43	31	-1	AUUUAUUUCAGUACCCUCCAUGGA	TTTT	597
Human-Exon 43	32	1	CUGAAAUAAAUUCUACAGUUCCCU	TTTC	598
Human-Exon 43	33	-1	UUUAUUUCAGUACCCUCCAUGGAA	TTTT	599
Human-Exon 43	34	-1	UACCCUCCAUGGAAAAAAGACAGG	TTTC	600
Human-Exon 43	35	-1	ACCCUCCAUGGAAAAAAGACAGGG	TTTT	601
Human-Exon 43	36	-1	CCCUCCAUGGAAAAAAGACAGGGA	TTTT	602
Human-Exon 43	37	1	UUUUUUCCAUGGAGGGUACUGAAA	TTTA	603

Human-Exon 43	38	1	UGUCUUUUUUCCAUGGAGGGUACU	TTTC	604
Human-Exon 6	1	1	CCUUGAGCAAGAACCAUGCAAACU	TTTA	605
Human-Exon 6	2	-1	UGCUCAAGGAAUGCAUUUUCUUAU	TTTC	606
Human-Exon 6	3	-1	GCUCAAGGAAUGCAUUUUCUUAUG	TTTT	607
Human-Exon 6	4	1	UGCAUUCCUUGAGCAAGAACCAUG	TTTG	608
Human-Exon 6	5	-1	GAAAAUUUAUUUCCACAUGUAGGU	TTTG	609
Human-Exon 6	6	-1	AAAAUUUAUUUCCACAUGUAGGUC	TTTT	610
Human-Exon 6	7	-1	AAAUUUAUUUCCACAUGUAGGUCA	TTTT	611
Human-Exon 6	8	1	CAUGUGGAAAUAAAUUUUCAUAAG	TTTT	612
Human-Exon 6	9	1	ACAUGUGGAAAUAAAUUUUCAUAA	TTTC	613
Human-Exon 6	10	-1	CCACAUGUAGGUCAAAAAUGUAAU	TTTC	614
Human-Exon 6	11	-1	CACAUGUAGGUCAAAAAUGUAAUG	TTTT	615
Human-Exon 6	12	-1	ACAUGUAGGUCAAAAAUGUAAUGA	TTTT	616
Human-Exon 6	13	1	ACAUUUUUGACCUACAUGUGGAAA	TTTA	617
Human-Exon 6	14	1	CAUUACAUUUUUGACCUACAUGUG	TTTC	618
Human-Exon 6	15	-1	AAAAAUAUCAUGGCUGGAUUGCAA	TTTG	619
Human-Exon 6	16	-1	GCUGGAUUGCAACAAACCAACAGU	TTTC	620
Human-Exon 6	17	-1	CUGGAUUGCAACAAACCAACAGUG	TTTT	621
Human-Exon 6	18	1	CCUAUGACUAUGGAUGAGAGCAUU	TTTG	622
Human-Exon 6	19	-1	UAGGUAAGAAGAUUACUGAGACAU	TTTA	623
Human-Exon 6	20	-1	AUUACUGAGACAUUAAAUAACUUG	TTTA	624
Human-Exon 6	21	-1	UUACUGAGACAUUAAAUAACUUGU	TTTT	625
Human-Exon 6	22	1	GGGGAAAAAUAUGUCAUCAGAGUC	TTTA	626
Human-Exon 6	23	1	CAUGAUCUGGAACCAUACUGGGGA	TTTT	627
Human-Exon 6	24	1	ACAUGAUCUGGAACCAUACUGGGG	TTTT	628
Human-Exon 6	25	1	GACAUGAUCUGGAACCAUACUGGG	TTTC	629
Human-Exon 7	1	1	uacacacauacacaAAGACAAAUA	TTTA	630
Human-Exon 7	2	1	uacacauacacacauacacaAAGA	TTTG	631
Human-Exon 7	3	1	aacacauacacauacacacauaca	TTtg	632
Human-Exon 7	4	1	AUUCCAGUCAAAUAGGUCUGGCCU	ttTT	633
Human-Exon 7	5	1	UAUUCCAGUCAAAUAGGUCUGGCC	tTTA	634
Human-Exon 7	6	1	GCUGGCAAACCACACUAUUCCAGU	TTTG	635
Human-Exon 7	7	1	AGUCGUUGUGUGGCUGACUGCUGG	TTTG	636

Human-Exon 7	8	-1	CGCCAGAUAUCAAUUAGGCAUAGA	TTTC	637
Human-Exon 7	9	-1	AAACUACUCGAUCCUGAAGGUUGG	TTTA	638
Human-Exon 7	10	1	CAUACUAAAAGCAGUGGUAGUCCA	TTTC	639
Human-Exon 7	11	1	GAAAACAUUAAACUCUACCAUACU	TTTT	640
Human-Exon 7	12	1	UGAAAACAUUAAACUCUACCAUAC	TTTA	641
Human-Exon 8	1	-1	UUGUUCAUUAUCCUUUUAGAGUCU	TTTG	642
Human-Exon 8	2	1	AAAGGAUAAUGAACAAAUCAAAGU	TTTA	643
Human-Exon 8	3	-1	UAUCCUUUUAGAGUCUCAAAUAUA	TTTC	644
Human-Exon 8	4	1	ACUCUAAAAGGAUAAUGAACAAAU	TTTG	645
Human-Exon 8	5	-1	UUUUAGAGUCUCAAAUAUAGAAAC	TTTG	646
Human-Exon 8	6	-1	UUUAGAGUCUCAAAUAUAGAAACC	TTTT	647
Human-Exon 8	7	-1	UUAGAGUCUCAAAUAUAGAAACCA	TTTT	648
Human-Exon 8	8	1	UUGAGACUCUAAAAGGAUAAUGAA	TTTG	649
Human-Exon 8	9	1	UUUGGUUUCUAUAUUUGAGACUCU	TTTT	650
Human-Exon 8	10	1	UUUUGGUUUCUAUAUUUGAGACUC	TTTA	651
Human-Exon 8	11	-1	AGCAUUGAAGCCAUCCAGGAAGUG	TTTC	652
Human-Exon 8	12	1	GCUUCAAUGCUCACUUGUUGAGGC	TTTT	653
Human-Exon 8	13	1	GGCUUCAAUGCUCACUUGUUGAGG	TTTG	654
Human-Exon 8	14	-1	AGUGGAAAUGUUGCCAAGGCCACC	TTTA	655
Human-Exon 8	15	-1	GUUGCCAAGGCCACCUAAAGUGAC	TTTA	656
Human-Exon 8	16	-1	GAAGAACAUUUUCAGUUACAUCAU	TTTG	657
Human-Exon 8	17	-1	AUCAAAUGCACUAUUCUCAACAGG	TTTA	658
Human-Exon 8	18	1	AUAGUGCAUUUGAUGAUGUAACUG	TTTT	659
Human-Exon 8	19	1	AAUAGUGCAUUUGAUGAUGUAACU	TTTC	660
Human-Exon 8	20	-1	ACUAUUCUCAACAGGUAAAGUGUG	TTTA	661
Human-Exon 8	21	1	UACCUAAAAAUGCAUAUAAAACAG	TTTT	662
Human-Exon 8	22	1	AUACCUAAAAAUGCAUAUAAAACA	TTTC	663
Human-Exon 8	23	1	CACGUAAUACCUAAAAAUGCAUAU	TTTT	664
Human-Exon 8	24	1	GCACGUAAUACCUAAAAAUGCAUA	TTTA	665
Human-Exon 8	25	1	auauauauGUGCACGUAAUACCUA	TTTT	666
Human-Exon 8	26	1	uauauauauGUGCACGUAAUACCU	TTTT	667
Human-Exon 8	27	1	auauauauauGUGCACGUAAUACC	TTTA	668
Human-Exon 55	1	-1	CUGCACAAUAUUAUAGUUGUUGCU	TTTA	669

Human-Exon 55	2	1	AUAAAAAGAGAAAGAUGGAGGAAC	TTTA	670
Human-Exon 55	3	1	CACCUAGUGAACUCCAUAAAAAGA	TTTC	671
Human-Exon 55	4	1	AUGGUGCACCUAGUGAACUCCAUA	TTTT	672
Human-Exon 55	5	1	AAUGGUGCACCUAGUGAACUCCAU	TTTT	673
Human-Exon 55	6	1	GAAUGGUGCACCUAGUGAACUCCA	TTTA	674
Human-Exon 55	7	1	GACCAAAUGUUCAGAUGCAAUUAU	TTTA	675
Human-Exon 55	8	1	UCGCUCACUCACCCUGCAAAGGAC	TTTG	676
Human-Exon 55	9	-1	AGUGAGCGAGAGGCUGCUUUGGAA	TTTC	677
Human-Exon 55	10	1	GCAGCCUCUCGCUCACUCACCCUG	TTTG	678
Human-Exon 55	11	1	UUGCAGUAAUCUAUGAGUUUCUUC	TTTG	679
Human-Exon 55	12	-1	CUGCAACAGUUCCCCCUGGACCUG	TTTC	680
Human-Exon 55	13	-1	UGCAACAGUUCCCCCUGGACCUGG	TTTT	681
Human-Exon 55	14	-1	UUUCUUGCCUGGCUUACAGAAGCU	TTTC	682
Human-Exon 55	15	1	UUUCAGCUUCUGUAAGCCAGGCAA	TTTC	683
Human-Exon 55	16	-1	GUCCUACAGGAUGCUACCCGUAAG	TTTC	684
Human-Exon 55	17	-1	GGCUCCUAGAAGACUCCAAGGGAG	TTTA	685
Human-Exon 55	18	-1	GCUCCUAGAAGACUCCAAGGGAGU	TTTT	686
Human-Exon 55	19	-1	CUCCAAGGGAGUAAAAGAGCUGAU	TTTC	687
Human-Exon 55	20	1	UGGAUCCACAAGAGUGCUAAAGCG	TTTC	688
Human-Exon 55	21	1	GUUCAAUUGGAUCCACAAGAGUGC	TTTA	689
Human-Exon 55	22	-1	UACUUGUAACUGACAAGCCAGGGA	TTTG	690
Human-Exon 55	23	-1	ACUUGUAACUGACAAGCCAGGGAC	TTTT	691
Human-Exon 55	24	-1	GUAACUGACAAGCCAGGGACAAAA	TTTG	692
Human-Exon 55	25	-1	UAACUGACAAGCCAGGGACAAAAC	TTTT	693
Human-Exon 55	26	1	UCCCUGGCUUGUCAGUUACAAGUA	TTTG	694
Human-G1-exon51		1	CAGAGUAACAGUCUGAGUAGGAGc	TTTA	695
Human-G2-exon51		1	uacuuuguuuagcaauacauggua	TTTC	696
Human-G3-exon51		-1	uggcucaaauuguuacucuucaau	TTTA	697
mouse-Exon23-G1		1	CUUUCAAagaacuuugcagagccu	TTTG	698
mouse-Exon23-G2		1	guugaaGCCAUUUUGUUGCUCUUU	TTTG	699
mouse-Exon23-G3		1	guugaaGCCAUUUUAUUGCUCUUU	TTTG	700
mouse-Exon23-G4		-1	uuuugagGCUCUGCAAAGUUCUUU	TTTC	701

mouse-Exon23-G5		-1	aguuauuaaugcauagauauucag	TTTA	702
mouse-Exon23-G6		-1	uauaauaugcccuguaauauaaua	TTTC	703
mouse-Exon23-G7		1	uaaaggccaaaccucggcuuaccU	TTTC	704
mouse-Exon23-G8		1	ucaauaucuuugaaggacucuggg	TTTA	705

* In this table, upper case letters represent sgRNA nucleotides that align to the exon sequence of the gene. Lower case letters represent sgRNA nucleotides that align to the intron sequence of the gene.

Table 8: Genomic target sites for sgRNA in mouse *Dmd* Exon 51

ID sgRNA	Strand	Target site	SEQ ID NO:	PAM
Ex51-SA1	3'	AGAGTAACAGTCTGACTGG	706	CAG
Ex51-SD	5'	GAAATGATCATCAAACAGA	707	AGG
Ex51-SA-2	3'	CACTAGAGTAACAGTCTGAC	708	TGG

Table 9: gRNA sequences targeting mouse *Dmd* Exon 51

ID sgRNA	Strand	Target site	SEQ ID NO:	PAM
Ex51-SA1	3'	CCAGUCAGACUGUUACUCU	709	CAG
Ex51-SD	5'	UCUGUUUGAUGAUCAUUUC	710	AGG
Ex51-SA-2	3'	GUCAGACUGUUACUCUAGUG	711	TGG

Table 10: Genomic target sequences for sgRNAs targeting human *Dmd* Exon 51

ID sgRNA	Strand	Target site	SEQ ID NO:	PAM
Ex51-SA	3'	AGAGTAACAGTCTGAGTAG	712	GAG

Ex51-SD	5’	GAGATGATCATCAAGCAGA	713	AGG
Ex51-SA-2	3’	CACCAGAGTAACAGTCTGAG	714	TAG

Table 11: sgRNA sequences targeting human *Dmd* Exon 51

ID sgRNA	Strand	Target site	SEQ ID NO:	PAM
Ex51-SA	3’	CUACUCAGACUGUUACUCU	715	GAG
Ex51-SD	5’	UCUGCUUGAUGAUCAUCUC	716	AGG
Ex51-SA-2	3’	CUCAGACUGUUACUCUGGUG	717	TAG

Table 12: Genomic target sequences for sgRNAs targeting sites in various human *Dmd* Exons

ID sgRNA	Strand	Target site	SEQ ID NO:	PAM
Exon51-#1	3’	CAGAGTAACAGTCTGAGTAG	947	GAG
Exon51-#2	3’	CACCAGAGTAACAGTCTGAG	718	TAG
Exon51-#3	3’	TATTTTGGGTTTTTGCAAAA	719	AGG
Exon51-#4	3’	AGTAGGAGCTAAAATATTTT	720	GGG
Exon51-#5	3’	GAGTAGGAGCTAAAATATTT	721	TGG
Exon51-#6	3’	ACCAGAGTAACAGTCTGAGT	722	AGG
Exon51-#7	5’	TCCTACTCAGACTGTTACTC	723	TGG
Exon51-#8	5’	TACTCTGGTGACACAACCTG	724	TGG
Exon51-#9	3’	GCAGTTTCCTTAGTAACCAC	725	AGG
Exon51-#10	5’	GACACAACCTGTGGTTACTA	726	AGG
Exon51-#11	3’	TGTCACCAGAGTAACAGTCT	727	GAG
Exon51-#12	3’	AGGTTGTGTCACCAGAGTAA	728	CAG
Exon51-#13	3’	AACCACAGGTTGTGTCACCA	729	GAG

Exon51-#14	3'	GTAACCACAGGTTGTGTCAC	730	CAG
Exon53-#1	5'	ATTTATTTTTCCTTTTATTC	731	TAG
Exon53-#2	5'	TTTCCTTTTATTCTAGTTGA	732	AAG
Exon53-#3	3'	TGATTCTGAATTCTTTCAAC	733	TAG
Exon53-#4	3'	AATTCTTTCAACTAGAATAA	734	AAG
Exon53-#6	5'	TTATTCTAGTTGAAAGAATT	735	CAG
Exon53-#7	5'	TAGTTGAAAGAATTCAGAAT	736	CAG
Exon53-#8	5'	AATTCAGAATCAGTGGGATG	737	AAG
Exon53-#9	3'	ATTCTTTCAACTAGAATAAA	738	AGG
Exon53-#10	5'	TTGAAAGAATTCAGAATCAG	739	TGG
Exon53-#11	5'	TGAAAGAATTCAGAATCAGT	740	GGG
Exon53-#12	3'	ACTGTTGCCTCCGGTTCTGA	741	AGG
Exon44-#1	3'	CAGATCTGTCAAATCGCCTG	742	CAG
Exon44-#2	3'	AAAACGCCGCCATTTCTCAA	743	CAG
Exon44-#3	3'	AGATCTGTCAAATCGCCTGC	744	AGG
Exon44-#4	3'	TATGGATCAAGAAAAATAGA	745	TGG
Exon44-#5	3'	CGCCTGCAGGTAAAAGCATA	746	TGG
Exon44-#6	5'	ATCCATATGCTTTTACCTGC	747	AGG
Exon44-#8	5'	TTGACAGATCTGTTGAGAAA	748	TGG
Exon44-#9	5'	ACAGATCTGTTGAGAAATGG	749	CGG
Exon44-#11	5'	GGCGATTTGACAGATCTGTT	750	GAG
Exon44-#13	5'	GGCGTTTTCATTATGATATA	751	AAG
Exon44-#14	5'	ATGATATAAAGATATTTAAT	752	CAG
Exon44-#15	5'	GATATTTAATCAGTGGCTAA	753	CAG
Exon44-#16	5'	ATTTAATCAGTGGCTAACAG	754	AAG
Exon44-#17	3'	AGAAACTGTTCAGCTTCTGT	755	TAG

Exon43-#1	5'	GTTTTAAAATTTTTATATTA	756	CAG
Exon43-#2	5'	TTTTATATTACAGAATATAA	757	AAG
Exon43-#3	5'	ATATTACAGAATATAAAAGA	758	TAG
Exon45-#1	3'	GTTCCTGTAAGATACCAAAA	759	AGG
Exon45-#2	5'	TTGCCTTTTTGGTATCTTAC	760	AGG
Exon45-#3	5'	TGGTATCTTACAGGAACTCC	761	AGG
Exon45-#4	5'	ATCTTACAGGAACTCCAGGA	762	TGG
Exon45-#5	3'	GCCGCTGCCCAATGCCATCC	763	TGG
Exon45-#6	5'	CAGGAACTCCAGGATGGCAT	764	TGG
Exon45-#7	5'	AGGAACTCCAGGATGGCATT	765	GGG
Exon45-#8	5'	TCCAGGATGGCATTGGGCAG	766	CGG
Exon45-#9	5'	GTCAGAACATTGAATGCAAC	767	TGG
Exon45-#10	3'	AGTTCCTGTAAGATACCAAA	768	AAG
Exon45-#11	3'	TGCCATCCTGGAGTTCCTGT	769	AAG
Exon45-#12	5'	TTGGTATCTTACAGGAACTC	770	CAG
Exon45-#13	3'	CGCTGCCCAATGCCATCCTG	771	GAG
Exon45-#14	5'	AACTCCAGGATGGCATTGGG	772	CAG
Exon45-#15	5'	GGGCAGCGGCAAACTGTTGT	773	CAG
Exon52-#1	3'	AGATCTGTCAAATCGCCTGC	774	AGG
Exon52-#2	3'	AATCCTGCATTGTTGCCTGT	775	AAG
Exon52-#3	5'	CGCTGAAGAACCCTGATACT	776	AAG
Exon52-#4	3'	GAACAAATATCCCTTAGTAT	777	CAG
Exon52-#5	3'	CTGTAAGAACAAATATCCCT	778	TAG
Exon52-#6	5'	CTAAGGGATATTTGTTCTTA	779	CAG
Exon52-#8	5'	TGTTCTTACAGGCAACAATG	780	CAG
Exon52-#9	5'	CAACAATGCAGGATTTGGAA	781	CAG
Exon52-#10	5'	ACAATGCAGGATTTGGAACA	782	GAG

Exon52-#11	5'	ATTTGGAACAGAGGCGTCCC	783	CAG
Exon52-#12	5'	ACAGAGGCGTCCCCAGTTGG	784	AAG
Exon2-#1	5'	TATTTTTTTATTTTGCATTT	785	TAG
Exon2-#2	5'	TTATTTTGCATTTTAGATGA	786	AAG
Exon2-#3	5'	ATTTTGCATTTTAGATGAAA	787	GAG
Exon2-#4	5'	TTGCATTTTAGATGAAAGAG	788	AAG
Exon2-#5	5'	ATGAAAGAGAAGATGTTCAA	789	AAG

Table 13: gRNA sequences for targeting sites in various human *Dmd* Exons

ID sgRNA	Strand	Target site	SEQ ID NO:	PAM
Exon51-#1	3'	CUACUCAGACUGUUACUCUG	790	GAG
Exon51-#2	3'	CUCAGACUGUUACUCUGGUG	791	TAG
Exon51-#3	3'	UUUUGCAAAAACCCAAAAUA	792	AGG
Exon51-#4	3'	AAAAUAUUUUAGCUCCUACU	793	GGG
Exon51-#5	3'	AAAUAUUUUAGCUCCUACUC	794	TGG
Exon51-#6	3'	ACUCAGACUGUUACUCUGGU	795	AGG
Exon51-#7	5'	GAGUAACAGUCUGAGUAGGA	796	TGG
Exon51-#8	5'	CAGGUUGUGUCACCAGAGUA	797	TGG
Exon51-#9	3'	GUGGUUACUAAGGAAACUGC	798	AGG
Exon51-#10	5'	UAGUAACCACAGGUUGUGUC	799	AGG
Exon51-#11	3'	AGACUGUUACUCUGGUGACA	800	GAG
Exon51-#12	3'	UUACUCUGGUGACACAACCU	801	CAG
Exon51-#13	3'	UGGUGACACAACCUGUGGUU	802	GAG
Exon51-#14	3'	GUGACACAACCUGUGGUUAC	803	CAG
Exon53-#1	5'	GAAUAAAAGGAAAAAUAAAU	804	TAG
Exon53-#2	5'	UCAACUAGAAUAAAAGGAAA	805	AAG

Exon53-#3	3'	GUUGAAAGAAUUCAGAAUCA	806	TAG
Exon53-#4	3'	UUAUUCUAGUUGAAAGAAUU	807	AAG
Exon53-#6	5'	AAUUCUUUCAACUAGAAUAA	808	CAG
Exon53-#7	5'	AUUCUGAAUUCUUUCAACUA	809	CAG
Exon53-#8	5'	CAUCCCACUGAUUCUGAAUU	810	AAG
Exon53-#9	3'	UUUAUUCUAGUUGAAAGAAU	811	AGG
Exon53-#10	5'	CUGAUUCUGAAUUCUUUCAA	812	TGG
Exon53-#11	5'	ACUGAUUCUGAAUUCUUUCA	813	GGG
Exon53-#12	3'	UCAGAACCGGAGGCAACAGU	814	AGG
Exon44-#1	3'	CAGGCGAUUUGACAGAUCUG	815	CAG
Exon44-#2	3'	UUGAGAAAUGGCGGCGUUUU	816	CAG
Exon44-#3	3'	GCAGGCGAUUUGACAGAUCU	817	AGG
Exon44-#4	3'	UCUAUUUUUCUUGAUCCAUA	818	TGG
Exon44-#5	3'	UAUGCUUUUACCUGCAGGCG	819	TGG
Exon44-#6	5'	GCAGGUAAAAGCAUAUGGAU	820	AGG
Exon44-#8	5'	UUUCUCAACAGAUCUGUCAA	821	TGG
Exon44-#9	5'	CCAUUUCUCAACAGAUCUGU	822	CGG
Exon44-#11	5'	AACAGAUCUGUCAAAUCGCC	823	GAG
Exon44-#13	5'	UAUAUCAUAAUGAAAACGCC	824	AAG
Exon44-#14	5'	AUUAAAUAUCUUUAUAUCAU	825	CAG
Exon44-#15	5'	UUAGCCACUGAUUAAAUAUC	826	CAG
Exon44-#16	5'	CUGUUAGCCACUGAUUAAAU	827	AAG
Exon44-#17	3'	ACAGAAGCUGAACAGUUUCU	828	TAG
Exon43-#1	5'	UAAUAUAAAAAUUUUAAAAC	829	CAG
Exon43-#2	5'	UUAUAUUCUGUAAUAUAAAA	830	AAG
Exon43-#3	5'	UCUUUUAUAUUCUGUAAUAU	831	TAG

Exon45-#1	3'	UUUUGGUAUCUUACAGGAAC	832	AGG
Exon45-#2	5'	GUAAGAUACCAAAAAGGCAA	833	AGG
Exon45-#3	5'	GGAGUUCCUGUAAGAUACCA	834	AGG
Exon45-#4	5'	UCCUGGAGUUCCUGUAAGAU	835	TGG
Exon45-#5	3'	GGAUGGCAUUGGGCAGCGGC	836	TGG
Exon45-#6	5'	AUGCCAUCCUGGAGUUCCUG	837	TGG
Exon45-#7	5'	AAUGCCAUCCUGGAGUUCCU	838	GGG
Exon45-#8	5'	CUGCCCAAUGCCAUCCUGGA	839	CGG
Exon45-#9	5'	GUUGCAUUCAAUGUUCUGAC	840	TGG
Exon45-#10	3'	UUUGGUAUCUUACAGGAACU	841	AAG
Exon45-#11	3'	ACAGGAACUCCAGGAUGGCA	842	AAG
Exon45-#12	5'	GAGUUCCUGUAAGAUACCAA	843	CAG
Exon45-#13	3'	CAGGAUGGCAUUGGGCAGCG	844	GAG
Exon45-#14	5'	CCCAAUGCCAUCCUGGAGUU	845	CAG
Exon45-#15	5'	ACAACAGUUUGCCGCUGCCC	846	CAG
Exon52-#1	3'	GCAGGCGAUUUGACAGAUCU	847	AGG
Exon52-#2	3'	ACAGGCAACAAUGCAGGAUU	848	AAG
Exon52-#3	5'	AGUAUCAGGGUUCUUCAGCG	849	AAG
Exon52-#4	3'	AUACUAAGGGAUAUUUGUUC	850	CAG
Exon52-#5	3'	AGGGAUAUUUGUUCUUACAG	851	TAG
Exon52-#6	5'	UAAGAACAAAUAUCCCUUAG	852	CAG
Exon52-#8	5'	CAUUGUUGCCUGUAAGAACA	853	CAG
Exon52-#9	5'	UUCCAAAUCCUGCAUUGUUG	854	CAG
Exon52-#10	5'	UGUUCCAAAUCCUGCAUUGU	855	GAG
Exon52-#11	5'	GGGACGCCUCUGUUCCAAAU	856	CAG
Exon52-#12	5'	CCAACUGGGGACGCCUCUGU	857	AAG
Exon2-#1	5'	ACAGAGGCGUCCCCAGUUGG	858	TAG

Exon2-#2	5'	UCAUCUAAAAUGCAAAAUAA	859	AAG
Exon2-#3	5'	UUUCAUCUAAAAUGCAAAAU	860	GAG
Exon2-#4	5'	CUCUUUCAUCUAAAAUGCAA	861	AAG
Exon2-#5	5'	UUGAACAUCUUCUCUUUCAU	862	AAG

Table 14: Genomic targeting sequence for sgRNAs targeting dog *Dmd* Exon 51

ID sgRNA	Strand	Target site	SEQ ID NO:	PAM
Ex51-SA-2	3'	CACCAGAGTAACAGTCTGAC	863	TGG

Table 15: gRNA sequence for targeting dog *Dmd* Exon 51

ID sgRNA	Strand	Target site	SEQ ID NO:	PAM
Ex51-SA-2	3'	GUCAGACUGUUACUCUGGUG	864	TGG

Table 16 – Exon 43 & 45 gRNA sequences

sgRNA ID	Sequence (5'-3')	SEQ ID NO.
Ex45-gRNA#3	CGCTGCCCAATGCCATCCTG	948
Ex45-gRNA#4	ATCTTACAGGAACTCCAGGA	949
Ex45-gRNA#5	AGGAACTCCAGGATGGCATT	950
Ex45-gRNA#6	CGCTGCCCAATGCCATCC	951
Ex43-gRNA#1	GTTTTAAAATTTTTATATTA	952
Ex43-gRNA#2	TTTTATATTACAGAATATAA	953
Ex43-gRNA#4	TATGTGTTACCTACCCTTGT	954
Ex43-gRNA#6	GTACAAGGACCGACAAGGGT	955

Table 17 – Exon 43 & 45 gRNA sequences

sgRNA ID	Sequence (5'-3')	SEQ ID NO.
Ex45-gRNA#3	CAGGAUGGCAUUGGGCAGCG	956
Ex45-gRNA#4	UCCUGGAGUUCCUGUAAGAU	957
Ex45-gRNA#5	AAUGCCAUCCUGGAGUUCCU	958
Ex45-gRNA#6	GGAUGGCAUUGGGCAGCG	959
Ex43-gRNA#1	UAAUAUAAAAAUUUUAAAAC	960
Ex43-gRNA#2	UUAUAUUCUGUAAUAUAAAA	961
Ex43-gRNA#4	ACAAGGGUAGGUAACACAUA	962
Ex43-gRNA#6	ACCCUUGUCGGUCCUUGUAC	963
Ex45-gRNA#3'	CGCUGCCCAAUGCCAUCCUG	964
Ex45-gRNA#4'	AUCUUACAGGAACUCCAGGA	965
Ex45-gRNA#5'	AGGAACUCCAGGAUGGCAUU	966
Ex45-gRNA#6'	CGCUGCCCAAUGCCAUCC	967
Ex43-gRNA#1'	GUUUUAAAAUUUUUAUAUUA	968
Ex43-gRNA#2'	UUUUAUAUUACAGAAUAUAA	969
Ex43-gRNA#4'	UAUGUGUUACCUACCCUUGU	970
Ex43-gRNA#6'	GUACAAGGACCGACAAGGGU	971

TABLE 18 – gRNA sequences

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 51	4	1	tttt	tctttttcttctttttttcctttttt	972	ucuuuuucuucuuuuuuccuuuuu	1305
Human-Exon 51	5	1	tttt	ctttttcttctttttttcctttttG	973	cuuuuucuucuuuuuuccuuuuuG	1306
Human-Exon 51	6	1	tttc	ttttttcttctttttttcctttttGC	974	uuuuucuucuuuuuuccuuuuuGC	1307
Human-Exon 51	7	1	tttt	tcttctttttttcctttttGCAAAA	975	ucuucuuuuuuccuuuuuGCAAAA	1308
Human-Exon 51	8	1	tttt	cttctttttttcctttttGCAAAAA	976	cuucuuuuuuccuuuuuGCAAAAA	1309
Human-Exon 51	9	1	tttc	ttctttttttcctttttGCAAAAAC	977	uucuuuuuuccuuuuuGCAAAAAC	1310
Human-Exon 51	10	1	tttt	ttcctttttGCAAAAACCCAAAAT	978	uuccuuuuuGCAAAAACCCAAAAU	1311
Human-Exon 51	11	1	tttt	tcctttttGCAAAAACCCAAAATA	979	uccuuuuuGCAAAAACCCAAAAUA	1312
Human-Exon 51	12	1	tttt	cctttttGCAAAAACCCAAAATAT	980	ccuuuuuGCAAAAACCCAAAAUAU	1313
Human-Exon 51	13	1	tttc	cttttGCAAAAACCCAAAATATT	981	cuuuuuGCAAAAACCCAAAAUAUU	1314
Human-Exon 51	14	1	tttt	tGCAAAAACCCAAAATATTTTAGC	982	uGCAAAAACCCAAAAUAUUUUAGC	1315
Human-Exon 51	15	1	tttt	GCAAAAACCCAAAATATTTTAGCT	983	GCAAAAACCCAAAAUAUUUUAGCU	1316
Human-Exon 51	16	1	tttG	CAAAAACCCAAAATATTTTAGCTC	984	CAAAAACCCAAAAUAUUUUAGCUC	1317
Human-Exon 51	17	1	TTTT	AGCTCCTACTCAGACTGTTACTCT	985	AGCUCCUACUCAGACUGUUACUCU	1318
Human-Exon 51	18	1	TTTA	GCTCCTACTCAGACTGTTACTCTG	986	GCUCCUACUCAGACUGUUACUCUG	1319

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 51	19	-1	TTTC	CTTAGTAACCACAGGTTGTGTCAC	987	CUUAGUAACCACAGGUUGUGUCAC	1320
Human-Exon 51	20	-1	TTTG	GAGATGGCAGTTTCCTTAGTAACC	988	GAGAUGGCAGUUUCCUUAGUAACC	1321
Human-Exon 51	21	-1	TTTC	TAGTTTGGAGATGGCAGTTTCCTT	999	UAGUUUGGAGAUGGCAGUUUCCUU	1322
Human-Exon 51	22	-1	TTTT	TTCTCATACCTTCTGCTTGATGAT	1000	UUCUCAUACCUUCUGCUUGAUGAU	1323
Human-Exon 51	23	-1	TTTA	TCATTTTTTCTCATACCTTCTGCT	1001	UCAUUUUUUCUCAUACCUUCUGCU	1324
Human-Exon 51	24	-1	TTTT	ATCATTTTTTCTCATACCTTCTGC	1002	AUCAUUUUUUCUCAUACCUUCUGC	1325
Human-Exon 51	25	-1	TTTA	AAGAAAAACTTCTGCCAACTTTTA	1003	AAGAAAAACUUCUGCCAACUUUUA	1326
Human-Exon 51	26	-1	TTTT	AAAGAAAAACTTCTGCCAACTTTT	1004	AAAGAAAAACUUCUGCCAACUUUU	1327
Human-Exon 51	27	1	TTTT	TCTTTAAAATGAAGATTTTCCACC	1005	UCUUUAAAAUGAAGAUUUUCCACC	1328
Human-Exon 51	28	1	TTTT	CTTTAAAATGAAGATTTTCCACCA	1006	CUUUAAAAUGAAGAUUUUCCACCA	1329
Human-Exon 51	29	1	TTTC	TTTAAAATGAAGATTTTCCACCAA	1007	UUUAAAAUGAAGAUUUUCCACCAA	1330
Human-Exon 51	30	1	TTTA	AAATGAAGATTTTCCACCAATCAC	1008	AAAUGAAGAUUUUCCACCAAUCAC	1331
Human-Exon 51	31	1	TTTT	CCACCAATCACTTTACTCTCCTAG	1009	CCACCAAUCACUUUACUCUCCUAG	1332
Human-Exon 51	32	1	TTTC	CACCAATCACTTTACTCTCCTAGA	1010	CACCAAUCACUUUACUCUCCUAGA	1333
Human-Exon 51	33	1	TTTA	CTCTCCTAGACCATTTCCCACCAG	1011	CUCUCCUAGACCAUUUCCCACCAG	1334
Human-Exon 45	1	-1	tttg	agaaaagattaaacagtgtgctac	1012	agaaaagauuaaacagugugcuac	1335
Human-Exon 45	2	-1	TTTa	tttgagaaaagattaaacagtgtg	1013	uuugagaaaagauuaaacagugug	1336

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 45	3	-1	TTTT	atttgagaaaagattaaacagtgt	1014	auuugagaaaagauuaaacagugu	1337
Human-Exon 45	4	-1	TTTT	Tatttgagaaaagattaaacagtg	1015	Uauuugagaaaagauuaaacagug	1338
Human-Exon 45	5	1	ttta	atcttttctcaaatAAAAAGACAT	1016	aucuuuucucaaauAAAAAGACAU	1339
Human-Exon 45	6	1	tttt	ctcaaatAAAAAGACATGGGGCTT	1017	cucaaauAAAAAGACAUGGGGCUU	1340
Human-Exon 45	7	1	tttc	tcaaatAAAAAGACATGGGGCTTC	1018	ucaaauAAAAAGACAUGGGGCUUC	1341
Human-Exon 45	8	1	TTTT	TGTTTTGCCTTTTTGGTATCTTAC	1019	UGUUUUGCCUUUUUGGUAUCUUAC	1342
Human-Exon 45	9	1	TTTT	GTTTTGCCTTTTTGGTATCTTACA	1020	GUUUUGCCUUUUUGGUAUCUUACA	1343
Human-Exon 45	10	1	TTTG	TTTTGCCTTTTTGGTATCTTACAG	1021	UUUUGCCUUUUUGGUAUCUUACAG	1344
Human-Exon 45	11	1	TTTT	GCCTTTTTGGTATCTTACAGGAAC	1022	GCCUUUUUGGUAUCUUACAGGAAC	1345
Human-Exon 45	12	1	TTTG	CCTTTTTGGTATCTTACAGGAACT	1023	CCUUUUUGGUAUCUUACAGGAACU	1346
Human-Exon 45	13	1	TTTT	TGGTATCTTACAGGAACTCCAGGA	1024	UGGUAUCUUACAGGAACUCCAGGA	1347
Human-Exon 45	14	1	TTTT	GGTATCTTACAGGAACTCCAGGAT	1025	GGUAUCUUACAGGAACUCCAGGAU	1348
Human-Exon 45	15	-1	TTTG	AGGATTGCTGAATTATTTCTTCCC	1026	AGGAUUGCUGAAUUAUUUCUUCCC	1349
Human-Exon 45	16	-1	TTTT	GAGGATTGCTGAATTATTTCTTCC	1027	GAGGAUUGCUGAAUUAUUUCUUCC	1350
Human-Exon 45	17	-1	TTTT	TGAGGATTGCTGAATTATTTCTTC	1028	UGAGGAUUGCUGAAUUAUUUCUUC	1351
Human-Exon 45	18	-1	TTTC	CTGTAGAATACTGGCATCTGTTTT	1029	CUGUAGAAUACUGGCAUCUGUUUU	1352
Human-Exon 45	19	-1	TTTT	CCTGTAGAATACTGGCATCTGTTT	1030	CCUGUAGAAUACUGGCAUCUGUUU	1353

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 45	20	-1	TTTT	TCCTGTAGAATACTGGCATCTGTT	1031	UCCUGUAGAAUACUGGCAUCUGUU	1354
Human-Exon 45	21	-1	TTTG	CAGACCTCCTGCCACCGCAGATTC	1032	CAGACCUCCUGCCACCGCAGAUUC	1355
Human-Exon 45	22	-1	TTTC	TGTCTGACAGCTGTTTGCAGACCT	1033	UGUCUGACAGCUGUUUGCAGACCU	1356
Human-Exon 45	23	-1	TTTT	CTGTCTGACAGCTGTTTGCAGACC	1034	CUGUCUGACAGCUGUUUGCAGACC	1357
Human-Exon 45	24	-1	TTTT	TCTGTCTGACAGCTGTTTGCAGAC	1035	UCUGUCUGACAGCUGUUUGCAGAC	1358
Human-Exon 45	25	-1	TTTT	TTCTGTCTGACAGCTGTTTGCAGA	1036	UUCUGUCUGACAGCUGUUUGCAGA	1359
Human-Exon 45	26	-1	TTTC	ATTCCTATTAGATCTGTCGCCCTA	1037	AUUCCUAUUAGAUCUGUCGCCCUA	1360
Human-Exon 45	27	-1	TTTT	CATTCCTATTAGATCTGTCGCCCT	1038	CAUUCCUAUUAGAUCUGUCGCCCU	1361
Human-Exon 45	28	1	TTTT	AGCAGACTTTTTAAGCTTTCTTTA	1039	AGCAGACUUUUUAAGCUUUCUUUA	1362
Human-Exon 45	29	1	TTTA	GCAGACTTTTTAAGCTTTCTTTAG	1040	GCAGACUUUUUAAGCUUUCUUUAG	1363
Human-Exon 45	30	1	TTTT	TAAGCTTTCTTTAGAAGAATATTT	1041	UAAGCUUUCUUUAGAAGAAUAUUU	1364
Human-Exon 45	31	1	TTTT	AAGCTTTCTTTAGAAGAATATTTC	1042	AAGCUUUCUUUAGAAGAAUAUUUC	1365
Human-Exon 45	32	1	TTTA	AGCTTTCTTTAGAAGAATATTTCA	1043	AGCUUUCUUUAGAAGAAUAUUUCA	1366
Human-Exon 45	33	1	TTTC	TTTAGAAGAATATTTCATGAGAGA	1044	UUUAGAAGAAUAUUUCAUGAGAGA	1367
Human-Exon 45	34	1	TTTA	GAAGAATATTTCATGAGAGATTAT	1045	GAAGAAUAUUUCAUGAGAGAUUAU	1368
Human-Exon 44	1	1	TTTG	TCAGTATAACCAAAAAATATACGC	1046	UCAGUAUAACCAAAAAAUAUACGC	1369
Human-Exon 44	2	1	tttt	acataatccatctatttttcttga	1047	acauaauccaucuauuuuucuuga	1370

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Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 44	3	1	ttta	cataatccatctatttttcttgat	1048	cauaauccaucuauuuuucuugau	1371
Human-Exon 44	4	1	tttt	tcttgatccatatgcttttACCTG	1049	ucuugauccauaugcuuuuACCUG	1372
Human-Exon 44	5	1	tttt	cttgatccatatgcttttACCTGC	1050	cuugauccauaugcuuuuACCUGC	1373
Human-Exon 44	6	1	tttc	ttgatccatatgcttttACCTGCA	1051	uugauccauaugcuuuuACCUGCA	1374
Human-Exon 44	7	-1	TTTC	TCAACAGATCTGTCAAATCGCCTG	1052	UCAACAGAUCUGUCAAAUCGCCUG	1375
Human-Exon 44	8	1	tttt	ACCTGCAGGCGATTTGACAGATCT	1053	ACCUGCAGGCGAUUUGACAGAUCU	1376
Human-Exon 44	9	1	tttA	CCTGCAGGCGATTTGACAGATCTG	1054	CCUGCAGGCGAUUUGACAGAUCUG	1377
Human-Exon 44	10	1	TTTG	ACAGATCTGTTGAGAAATGGCGGC	1055	ACAGAUCUGUUGAGAAAUGGCGGC	1378
Human-Exon 44	11	-1	TTTA	TATCATAATGAAAACGCCGCCATT	1056	UAUCAUAAUGAAAACGCCGCCAUU	1379
Human-Exon 44	12	1	TTTT	CATTATGATATAAAGATATTTAAT	1057	CAUUAUGAUAUAAAGAUAUUUAAU	1380
Human-Exon 44	13	-1	TTTG	TATTTAGCATGTTCCCAATTCTCA	1058	UAUUUAGCAUGUUCCCAAUUCUCA	1381
Human-Exon 44	14	-1	TTTC	GAAAAAACAAATCAAAGACTTACC	1059	GAAAAAACAAAUCAAAGACUUACC	1382
Human-Exon 44	15	1	TTTG	ATTTGTTTTTTCGAAATTGTATTT	1060	AUUUGUUUUUUCGAAAUUGUAUUU	1383
Human-Exon 44	16	1	TTTG	TTTTTTCGAAATTGTATTTATCTT	1061	UUUUUUCGAAAUUGUAUUUAUCUU	1384
Human-Exon 44	17	1	TTTT	TTCGAAATTGTATTTATCTTCAGC	1062	UUCGAAAUUGUAUUUAUCUUCAGC	1385
Human-Exon 44	18	1	TTTT	TCGAAATTGTATTTATCTTCAGCA	1063	UCGAAAUUGUAUUUAUCUUCAGCA	1386
Human-Exon 44	19	1	TTTT	CGAAATTGTATTTATCTTCAGCAC	1064	CGAAAUUGUAUUUAUCUUCAGCAC	1387

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 44	20	1	TTTC	GAAATTGTATTTATCTTCAGCACA	1065	GAAAUUGUAUUUAUCUUCAGCACA	1388
Human-Exon 44	21	-1	TTTA	AGAAGTTAAAGAGTCCAGATGTGC	1066	AGAAGUUAAAGAGUCCAGAUGUGC	1389
Human-Exon 44	22	1	TTTA	TCTTCAGCACATCTGGACTCTTTA	1067	UCUUCAGCACAUCUGGACUCUUUA	1390
Human-Exon 44	23	-1	TTTC	CATCACCCTTCAGAACCTGATCTT	1068	CAUCACCCUUCAGAACCUGAUCUU	1391
Human-Exon 44	24	1	TTTA	ACTTCTTAAAGATCAGGTTCTGAA	1069	ACUUCUUAAAGAUCAGGUUCUGAA	1392
Human-Exon 44	25	1	TTTT	GACTGTTGTTGTCATCATTATATT	1070	GACUGUUGUUGUCAUCAUUAUAUU	1393
Human-Exon 44	26	1	TTTG	ACTGTTGTTGTCATCATTATATTA	1071	ACUGUUGUUGUCAUCAUUAUAUUA	1394
Human-Exon 53	1	-1	TTTC	AACTAGAATAAAAGGAAAAATAAA	1072	AACUAGAAUAAAAGGAAAAAUAAA	1395
Human-Exon 53	2	1	TTTA	CTACTATATATTTATTTTTCCTTT	1073	CUACUAUAUAUUUAUUUUUCCUUU	1396
Human-Exon 53	3	1	TTTA	TTTTTCCTTTTATTCTAGTTGAAA	1074	UUUUUCCUUUUAUUCUAGUUGAAA	1397
Human-Exon 53	4	1	TTTT	TCCTTTTATTCTAGTTGAAAGAAT	1075	UCCUUUUAUUCUAGUUGAAAGAAU	1398
Human-Exon 53	5	1	TTTT	CCTTTTATTCTAGTTGAAAGAATT	1076	CCUUUUAUUCUAGUUGAAAGAAUU	1399
Human-Exon 53	6	1	TTTC	CTTTTATTCTAGTTGAAAGAATTC	1077	CUUUUAUUCUAGUUGAAAGAAUUC	1400
Human-Exon 53	7	1	TTTT	ATTCTAGTTGAAAGAATTCAGAAT	1078	AUUCUAGUUGAAAGAAUUCAGAAU	1401
Human-Exon 53	8	1	TTTA	TTCTAGTTGAAAGAATTCAGAATC	1079	UUCUAGUUGAAAGAAUUCAGAAUC	1402
Human-Exon 53	9	-1	TTTC	ATTCAACTGTTGCCTCCGGTTCTG	1080	AUUCAACUGUUGCCUCCGGUUCUG	1403
Human-Exon 53	10	-1	TTTA	ACATTTCATTCAACTGTTGCCTCC	1081	ACAUUUCAUUCAACUGUUGCCUCC	1404

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 53	11	-1	TTTT	CTTTTGGATTGCATCTACTGTATA	1082	CUUUUGGAUUGCAUCUACUGUAUA	1405
Human-Exon 53	12	-1	TTTC	TGTGATTTTCTTTTGGATTGCATC	1083	UGUGAUUUUCUUUUGGAUUGCAUC	1406
Human-Exon 53	13	-1	TTTG	ATACTAACCTTGGTTTCTGTGATT	1084	AUACUAACCUUGGUUUCUGUGAUU	1407
Human-Exon 53	14	-1	TTTA	AAAAGGTATCTTTGATACTAACCT	1085	AAAAGGUAUCUUUGAUACUAACCU	1408
Human-Exon 53	15	-1	TTTT	AAAAAGGTATCTTTGATACTAACC	1086	AAAAAGGUAUCUUUGAUACUAACC	1409
Human-Exon 53	16	-1	TTTA	TTTTAAAAAGGTATCTTTGATACT	1087	UUUUAAAAAGGUAUCUUUGAUACU	1410
Human-Exon 53	17	-1	TTTT	ATTTTAAAAAGGTATCTTTGATAC	1088	AUUUUAAAAAGGUAUCUUUGAUAC	1411
Human-Exon 46	1	-1	TTTG	TTAATGCAAACTGGGACACAAACA	1089	UUAAUGCAAACUGGGACACAAACA	1412
Human-Exon 46	2	1	TTTT	TAAATTGCCATGTTTGTGTCCCAG	1090	UAAAUUGCCAUGUUUGUGUCCCAG	1413
Human-Exon 46	3	1	TTTT	AAATTGCCATGTTTGTGTCCCAGT	1091	AAAUUGCCAUGUUUGUGUCCCAGU	1414
Human-Exon 46	4	1	TTTA	AATTGCCATGTTTGTGTCCCAGTT	1092	AAUUGCCAUGUUUGUGUCCCAGUU	1415
Human-Exon 46	5	1	TTTG	TGTCCCAGTTTGCATTAACAAATA	1093	UGUCCCAGUUUGCAUUAACAAAUA	1416
Human-Exon 46	6	-1	tttC	CAACATAGTTCTCAAACTATTTGT	1094	CAACAUAGUUCUCAAACUAUUUGU	1417
Human-Exon 46	7	-1	tttt	CCAACATAGTTCTCAAACTATTTG	1095	CCAACAUAGUUCUCAAACUAUUUG	1418
Human-Exon 46	8	-1	tttt	tCCAACATAGTTCTCAAACTATTT	1096	uCCAACAUAGUUCUCAAACUAUUU	1419
Human-Exon 46	9	-1	tttt	tttCCAACATAGTTCTCAAACTAT	1097	uuuCCAACAUAGUUCUCAAACUAU	1420
Human-Exon 46	10	-1	tttt	ttttCCAACATAGTTCTCAAACTA	1098	uuuuCCAACAUAGUUCUCAAACUA	1421

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 46	11	-1	tttt	tttttCCAACATAGTTCTCAAACT	1099	uuuuuCCAACAUAGUUCUCAAACU	1422
Human-Exon 46	12	1	TTTG	CATTAACAAATAGTTTGAGAACTA	1100	CAUUAACAAAUAGUUUGAGAACUA	1423
Human-Exon 46	13	1	TTTG	AGAACTATGTTGGaaaaaaaaTA	1101	AGAACUAUGUUGGaaaaaaaaUA	1424
Human-Exon 46	14	-1	TTTT	GTTCTTCTAGCCTGGAGAAAGAAG	1102	GUUCUUCUAGCCUGGAGAAAGAAG	1425
Human-Exon 46	15	1	TTTT	ATTCTTCTTTCTCCAGGCTAGAAG	1103	AUUCUUCUUUCUCCAGGCUAGAAG	1426
Human-Exon 46	16	1	TTTA	TTCTTCTTTCTCCAGGCTAGAAGA	1104	UUCUUCUUUCUCCAGGCUAGAAGA	1427
Human-Exon 46	17	1	TTTC	TCCAGGCTAGAAGAACAAAAGAAT	1105	UCCAGGCUAGAAGAACAAAAGAAU	1428
Human-Exon 46	18	-1	TTTG	AAATTCTGACAAGATATTCTTTTG	1106	AAAUUCUGACAAGAUAUUCUUUUG	1429
Human-Exon 46	19	-1	TTTT	CTTTTAGTTGCTGCTCTTTTCCAG	1107	CUUUUAGUUGCUGCUCUUUUCCAG	1430
Human-Exon 46	20	-1	TTTG	AGAAAATAAAATTACCTTGACTTG	1108	AGAAAAUAAAAUUACCUUGACUUG	1431
Human-Exon 46	21	-1	TTTA	TGCAAGCAGGCCCTGGGGGATTTG	1109	UGCAAGCAGGCCCUGGGGGAUUUG	1432
Human-Exon 46	22	1	TTTT	ATTTTCTCAAATCCCCCAGGGCCT	1110	AUUUUCUCAAAUCCCCCAGGGCCU	1433
Human-Exon 46	23	1	TTTA	TTTTCTCAAATCCCCCAGGGCCTG	1111	UUUUCUCAAAUCCCCCAGGGCCUG	1434
Human-Exon 46	24	1	TTTT	CTCAAATCCCCCAGGGCCTGCTTG	1112	CUCAAAUCCCCCAGGGCCUGCUUG	1435
Human-Exon 46	25	1	TTTC	TCAAATCCCCCAGGGCCTGCTTGC	1113	UCAAAUCCCCCAGGGCCUGCUUGC	1436
Human-Exon 46	26	1	TTTT	TTAATTCAATCATTGGTTTTCTGC	1114	UUAAUUCAAUCAUUGGUUUUCUGC	1437
Human-Exon 46	27	1	TTTT	TAATTCAATCATTGGTTTTCTGCC	1115	UAAUUCAAUCAUUGGUUUUCUGCC	1438

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 46	28	1	TTTT	AATTCAATCATTGGTTTTCTGCCC	1116	AAUUCAAUCAUUGGUUUUCUGCCC	1439
Human-Exon 46	29	1	TTTA	ATTCAATCATTGGTTTTCTGCCCA	1117	AUUCAAUCAUUGGUUUUCUGCCCA	1440
Human-Exon 46	30	-1	TTTA	GCAAGGAACTATGAATAACCTAAT	1118	GCAAGGAACUAUGAAUAACCUAAU	1441
Human-Exon 46	31	1	TTTT	CTGCCCATTAGGTTATTCATAGTT	1119	CUGCCCAUUAGGUUAUUCAUAGUU	1442
Human-Exon 46	32	1	TTTC	TGCCCATTAGGTTATTCATAGTTC	1120	UGCCCAUUAGGUUAUUCAUAGUUC	1443
Human-Exon 52	1	-1	TTTA	TAGAAAACAATTTAACAGGAAATA	1121	UAGAAAACAAUUUAACAGGAAAUA	1444
Human-Exon 52	2	1	TTTC	CTGTTAAATTGTTTTCTATAAACC	1122	CUGUUAAAUUGUUUUCUAUAAACC	1445
Human-Exon 52	3	-1	TTTA	GAAATAAAAAAGATGTTACTGTAT	1123	GAAAUAAAAAAGAUGUUACUGUAU	1446
Human-Exon 52	4	-1	TTTT	AGAAATAAAAAAGATGTTACTGTA	1124	AGAAAUAAAAAAGAUGUUACUGUA	1447
Human-Exon 52	5	1	TTTT	CTATAAACCCTTATACAGTAACAT	1125	CUAUAAACCCUUAUACAGUAACAU	1448
Human-Exon 52	6	1	TTTC	TATAAACCCTTATACAGTAACATC	1126	UAUAAACCCUUAUACAGUAACAUC	1449
Human-Exon 52	7	1	TTTT	TTATTTCTAAAAGTGTTTTGGCTG	1127	UUAUUUCUAAAAGUGUUUUGGCUG	1450
Human-Exon 52	8	1	TTTT	TATTTCTAAAAGTGTTTTGGCTGG	1128	UAUUUCUAAAAGUGUUUUGGCUGG	1451
Human-Exon 52	9	1	TTTT	ATTTCTAAAAGTGTTTTGGCTGGT	1129	AUUUCUAAAAGUGUUUUGGCUGGU	1452
Human-Exon 52	10	1	TTTA	TTTCTAAAAGTGTTTTGGCTGGTC	1130	UUUCUAAAAGUGUUUUGGCUGGUC	1453
Human-Exon 52	11	1	TTTC	TAAAAGTGTTTTGGCTGGTCTCAC	1131	UAAAAGUGUUUUGGCUGGUCUCAC	1454
Human-Exon 52	12	-1	TTTA	CATAATACAAAGTAAAGTACAATT	1132	CAUAAUACAAAGUAAAGUACAAUU	1455

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 52	13	-1	TTTT	ACATAATACAAAGTAAAGTACAAT	1133	ACAUAAUACAAAGUAAAGUACAAU	1456
Human-Exon 52	14	1	TTTT	GGCTGGTCTCACAATTGTACTTTA	1134	GGCUGGUCUCACAAUUGUACUUUA	1457
Human-Exon 52	15	1	TTTG	GCTGGTCTCACAATTGTACTTTAC	1135	GCUGGUCUCACAAUUGUACUUUAC	1458
Human-Exon 52	16	1	TTTA	CTTTGTATTATGTAAAAGGAATAC	1136	CUUUGUAUUAUGUAAAAGGAAUAC	1459
Human-Exon 52	17	1	TTTG	TATTATGTAAAAGGAATACACAAC	1137	UAUUAUGUAAAAGGAAUACACAAC	1460
Human-Exon 52	18	1	TTTG	TTCTTACAGGCAACAATGCAGGAT	1138	UUCUUACAGGCAACAAUGCAGGAU	1461
Human-Exon 52	19	1	TTTG	GAACAGAGGCGTCCCCAGTTGGAA	1139	GAACAGAGGCGUCCCCAGUUGGAA	1462
Human-Exon 52	20	-1	TTTG	GGCAGCGGTAATGAGTTCTTCCAA	1140	GGCAGCGGUAAUGAGUUCUUCCAA	1463
Human-Exon 52	21	-1	TTTT	TCAAATTTTGGGCAGCGGTAATGA	1141	UCAAAUUUUGGGCAGCGGUAAUGA	1464
Human-Exon 52	22	1	TTTG	AAAAACAAGACCAGCAATCAAGAG	1142	AAAAACAAGACCAGCAAUCAAGAG	1465
Human-Exon 52	23	-1	TTTG	TGTGTCCCATGCTTGTTAAAAAAC	1143	UGUGUCCCAUGCUUGUUAAAAAAC	1466
Human-Exon 52	24	1	TTTT	TTAACAAGCATGGGACACACAAAG	1144	UUAACAAGCAUGGGACACACAAAG	1467
Human-Exon 52	25	1	TTTT	TAACAAGCATGGGACACACAAAGC	1145	UAACAAGCAUGGGACACACAAAGC	1468
Human-Exon 52	26	1	TTTT	AACAAGCATGGGACACACAAAGCA	1146	AACAAGCAUGGGACACACAAAGCA	1469
Human-Exon 52	27	1	TTTA	ACAAGCATGGGACACACAAAGCAA	1147	ACAAGCAUGGGACACACAAAGCAA	1470
Human-Exon 52	28	-1	TTTA	TTGAAACTTGTCATGCATCTTGCT	1148	UUGAAACUUGUCAUGCAUCUUGCU	1471
Human-Exon 52	29	-1	TTTT	ATTGAAACTTGTCATGCATCTTGC	1149	AUUGAAACUUGUCAUGCAUCUUGC	1472

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 52	30	-1	TTTT	TATTGAAACTTGTCATGCATCTTG	1150	UAUUGAAACUUGUCAUGCAUCUUG	1473
Human-Exon 52	31	1	TTTC	AATAAAAACTTAAGTTCATATATC	1151	AAUAAAAACUUAAGUUCAUAUAUC	1474
Human-Exon 50	1	-1	TTTG	GTGAATATATTATTGGATTTCTAT	1152	GUGAAUAUAUUAUUGGAUUUCUAU	1475
Human-Exon 50	2	-1	TTTG	AAGATAATTCATGAACATCTTAAT	1153	AAGAUAAUUCAUGAACAUCUUAAU	1476
Human-Exon 50	3	-1	TTTA	ACAGAAAAGCATACACATTACTTA	1154	ACAGAAAAGCAUACACAUUACUUA	1477
Human-Exon 50	4	1	TTTT	CTGTTAAAGAGGAAGTTAGAAGAT	1155	CUGUUAAAGAGGAAGUUAGAAGAU	1478
Human-Exon 50	5	1	TTTC	TGTTAAAGAGGAAGTTAGAAGATC	1156	UGUUAAAGAGGAAGUUAGAAGAUC	1479
Human-Exon 50	6	-1	TTTA	CCGCCTTCCACTCAGAGCTCAGAT	1157	CCGCCUUCCACUCAGAGCUCAGAU	1480
Human-Exon 50	7	-1	TTTG	CCCTCAGCTCTTGAAGTAAACGGT	1158	CCCUCAGCUCUUGAAGUAAACGGU	1481
Human-Exon 50	8	1	TTTA	CTTCAAGAGCTGAGGGCAAAGCAG	1159	CUUCAAGAGCUGAGGGCAAAGCAG	1482
Human-Exon 50	9	-1	TTTG	AACAAATAGCTAGAGCCAAAGAGA	1160	AACAAAUAGCUAGAGCCAAAGAGA	1483
Human-Exon 50	10	-1	TTTT	GAACAAATAGCTAGAGCCAAAGAG	1161	GAACAAAUAGCUAGAGCCAAAGAG	1484
Human-Exon 50	11	1	TTTG	GCTCTAGCTATTTGTTCAAAAGTG	1162	GCUCUAGCUAUUUGUUCAAAAGUG	1485
Human-Exon 50	12	1	TTTG	TTCAAAAGTGCAACTATGAAGTGA	1163	UUCAAAAGUGCAACUAUGAAGUGA	1486
Human-Exon 50	13	-1	TTTC	TCTCTCACCCAGTCATCACTTCAT	1164	UCUCUCACCCAGUCAUCACUUCAU	1487
Human-Exon 50	14	-1	TTTT	CTCTCTCACCCAGTCATCACTTCA	1165	CUCUCUCACCCAGUCAUCACUUCA	1488
Human-Exon 43	1	1	TTTG	tatatatatatatTTTTCTCTT	1166	uauauauauauauauUUUUCUCUU	1489

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Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 43	2	1	tTTT	TCTCTTTCTATAGACAGCTAATTC	1167	UCUCUUUCUAUAGACAGCUAAUUC	1490
Human-Exon 43	3	1	TTTT	CTCTTTCTATAGACAGCTAATTCA	1168	CUCUUUCUAUAGACAGCUAAUUCA	1491
Human-Exon 43	4	-1	TTTA	AAACAGTAAAAAAATGAATTAGCT	1169	AAACAGUAAAAAAAUGAAUUAGCU	1492
Human-Exon 43	5	1	TTTC	TCTTTCTATAGACAGCTAATTCAT	1170	UCUUUCUAUAGACAGCUAAUUCAU	1493
Human-Exon 43	6	-1	TTTT	AAAACAGTAAAAAAATGAATTAGC	1171	AAAACAGUAAAAAAAUGAAUUAGC	1494
Human-Exon 43	7	1	TTTC	TATAGACAGCTAATTCATTTTTTT	1172	UAUAGACAGCUAAUUCAUUUUUUU	1495
Human-Exon 43	8	-1	TTTA	TATTCTGTAATATAAAAATTTTAA	1173	UAUUCUGUAAUAUAAAAAUUUUAA	1496
Human-Exon 43	9	-1	TTTT	ATATTCTGTAATATAAAAATTTTA	1174	AUAUUCUGUAAUAUAAAAAUUUUA	1497
Human-Exon 43	10	1	TTTT	TTTACTGTTTTAAAATTTTTATAT	1175	UUUACUGUUUUAAAAUUUUUAUAU	1498
Human-Exon 43	11	1	TTTT	TTACTGTTTTAAAATTTTTATATT	1176	UUACUGUUUUAAAAUUUUUAUAUU	1499
Human-Exon 43	12	1	TTTT	TACTGTTTTAAAATTTTTATATTA	1177	UACUGUUUUAAAAUUUUUAUAUUA	1500
Human-Exon 43	13	1	TTTT	ACTGTTTTAAAATTTTTATATTAC	1178	ACUGUUUUAAAAUUUUUAUAUUAC	1501
Human-Exon 43	14	1	TTTA	CTGTTTTAAAATTTTTATATTACA	1179	CUGUUUUAAAAUUUUUAUAUUACA	1502
Human-Exon 43	15	1	TTTT	AAAATTTTTATATTACAGAATATA	1180	AAAAUUUUUAUAUUACAGAAUAUA	1503
Human-Exon 43	16	1	TTTA	AAATTTTTATATTACAGAATATAA	1181	AAAUUUUUAUAUUACAGAAUAUAA	1504
Human-Exon 43	17	-1	TTTG	TTGTAGACTATCTTTTATATTCTG	1182	UUGUAGACUAUCUUUUAUAUUCUG	1505
Human-Exon 43	18	1	TTTT	TATATTACAGAATATAAAAGATAG	1183	UAUAUUACAGAAUAUAAAAGAUAG	1506

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 43	19	1	TTTT	ATATTACAGAATATAAAAGATAGT	1184	AUAUUACAGAAUAUAAAAGAUAGU	1507
Human-Exon 43	20	1	TTTA	TATTACAGAATATAAAAGATAGTC	1185	UAUUACAGAAUAUAAAAGAUAGUC	1508
Human-Exon 43	21	-1	TTTG	CAATGCTGCTGTCTTCTTGCTATG	1186	CAAUGCUGCUGUCUUCUUGCUAUG	1509
Human-Exon 43	22	1	TTTC	CAATGGGAAAAAGTTAACAAAATG	1187	CAAUGGGAAAAAGUUAACAAAAUG	1510
Human-Exon 43	23	-1	TTTC	TGCAAGTATCAAGAAAAATATATG	1188	UGCAAGUAUCAAGAAAAAUAUAUG	1511
Human-Exon 43	24	1	TTTT	TCTTGATACTTGCAGAAATGATTT	1189	UCUUGAUACUUGCAGAAAUGAUUU	1512
Human-Exon 43	25	1	TTTT	CTTGATACTTGCAGAAATGATTTG	1190	CUUGAUACUUGCAGAAAUGAUUUG	1513
Human-Exon 43	26	1	TTTC	TTGATACTTGCAGAAATGATTTGT	1191	UUGAUACUUGCAGAAAUGAUUUGU	1514
Human-Exon 43	27	1	TTTG	TTTTCAGGGAACTGTAGAATTTAT	1192	UUUUCAGGGAACUGUAGAAUUUAU	1515
Human-Exon 43	28	-1	TTTC	CATGGAGGGTACTGAAATAAATTC	1193	CAUGGAGGGUACUGAAAUAAAUUC	1516
Human-Exon 43	29	-1	TTTT	CCATGGAGGGTACTGAAATAAATT	1194	CCAUGGAGGGUACUGAAAUAAAUU	1517
Human-Exon 43	30	1	TTTT	CAGGGAACTGTAGAATTTATTTCA	1195	CAGGGAACUGUAGAAUUUAUUUCA	1518
Human-Exon 43	31	-1	TTTT	TCCATGGAGGGTACTGAAATAAAT	1196	UCCAUGGAGGGUACUGAAAUAAAU	1519
Human-Exon 43	32	1	TTTC	AGGGAACTGTAGAATTTATTTCAG	1197	AGGGAACUGUAGAAUUUAUUUCAG	1520
Human-Exon 43	33	-1	TTTT	TTCCATGGAGGGTACTGAAATAAA	1198	UUCCAUGGAGGGUACUGAAAUAAA	1521
Human-Exon 43	34	-1	TTTC	CCTGTCTTTTTTCCATGGAGGGTA	1199	CCUGUCUUUUUUCCAUGGAGGGUA	1522
Human-Exon 43	35	-1	TTTT	CCCTGTCTTTTTTCCATGGAGGGT	1200	CCCUGUCUUUUUUCCAUGGAGGGU	1523

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Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 43	36	-1	TTTT	TCCCTGTCTTTTTTCCATGGAGGG	1201	UCCCUGUCUUUUUUCCAUGGAGGG	1524
Human-Exon 43	37	1	TTTA	TTTCAGTACCCTCCATGGAAAAAA	1202	UUUCAGUACCCUCCAUGGAAAAAA	1525
Human-Exon 43	38	1	TTTC	AGTACCCTCCATGGAAAAAAGACA	1203	AGUACCCUCCAUGGAAAAAAGACA	1526
Human-Exon 6	1	1	TTTA	AGTTTGCATGGTTCTTGCTCAAGG	1204	AGUUUGCAUGGUUCUUGCUCAAGG	1527
Human-Exon 6	2	-1	TTTC	ATAAGAAAATGCATTCCTTGAGCA	1205	AUAAGAAAAUGCAUUCCUUGAGCA	1528
Human-Exon 6	3	-1	TTTT	CATAAGAAAATGCATTCCTTGAGC	1206	CAUAAGAAAAUGCAUUCCUUGAGC	1529
Human-Exon 6	4	1	TTTG	CATGGTTCTTGCTCAAGGAATGCA	1207	CAUGGUUCUUGCUCAAGGAAUGCA	1530
Human-Exon 6	5	-1	TTTG	ACCTACATGTGGAAATAAATTTTC	1208	ACCUACAUGUGGAAAUAAAUUUUC	1531
Human-Exon 6	6	-1	TTTT	GACCTACATGTGGAAATAAATTTT	1209	GACCUACAUGUGGAAAUAAAUUUU	1532
Human-Exon 6	7	-1	TTTT	TGACCTACATGTGGAAATAAATTT	1210	UGACCUACAUGUGGAAAUAAAUUU	1533
Human-Exon 6	8	1	TTTT	CTTATGAAAATTTATTTCCACATG	1211	CUUAUGAAAAUUUAUUUCCACAUG	1534
Human-Exon 6	9	1	TTTC	TTATGAAAATTTATTTCCACATGT	1212	UUAUGAAAAUUUAUUUCCACAUGU	1535
Human-Exon 6	10	-1	TTTC	ATTACATTTTTGACCTACATGTGG	1213	AUUACAUUUUUGACCUACAUGUGG	1536
Human-Exon 6	11	-1	TTTT	CATTACATTTTTGACCTACATGTG	1214	CAUUACAUUUUUGACCUACAUGUG	1537
Human-Exon 6	12	-1	TTTT	TCATTACATTTTTGACCTACATGT	1215	UCAUUACAUUUUUGACCUACAUGU	1538
Human-Exon 6	13	1	TTTA	TTTCCACATGTAGGTCAAAAATGT	1216	UUUCCACAUGUAGGUCAAAAAUGU	1539
Human-Exon 6	14	1	TTTC	CACATGTAGGTCAAAAATGTAATG	1217	CACAUGUAGGUCAAAAAUGUAAUG	1540

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 6	15	-1	TTTG	TTGCAATCCAGCCATGATATTTTT	1218	UUGCAAUCCAGCCAUGAUAUUUUU	1541
Human-Exon 6	16	-1	TTTC	ACTGTTGGTTTGTTGCAATCCAGC	1219	ACUGUUGGUUUGUUGCAAUCCAGC	1542
Human-Exon 6	17	-1	TTTT	CACTGTTGGTTTGTTGCAATCCAG	1220	CACUGUUGGUUUGUUGCAAUCCAG	1543
Human-Exon 6	18	1	TTTG	AATGCTCTCATCCATAGTCATAGG	1221	AAUGCUCUCAUCCAUAGUCAUAGG	1544
Human-Exon 6	19	-1	TTTA	ATGTCTCAGTAATCTTCTTACCTA	1222	AUGUCUCAGUAAUCUUCUUACCUA	1545
Human-Exon 6	20	-1	TTTA	CAAGTTATTTAATGTCTCAGTAAT	1223	CAAGUUAUUUAAUGUCUCAGUAAU	1546
Human-Exon 6	21	-1	TTTT	ACAAGTTATTTAATGTCTCAGTAA	1224	ACAAGUUAUUUAAUGUCUCAGUAA	1547
Human-Exon 6	22	1	TTTA	GACTCTGATGACATATTTTTCCCC	1225	GACUCUGAUGACAUAUUUUUCCCC	1548
Human-Exon 6	23	1	TTTT	TCCCCAGTATGGTTCCAGATCATG	1226	UCCCCAGUAUGGUUCCAGAUCAUG	1549
Human-Exon 6	24	1	TTTT	CCCCAGTATGGTTCCAGATCATGT	1227	CCCCAGUAUGGUUCCAGAUCAUGU	1550
Human-Exon 6	25	1	TTTC	CCCAGTATGGTTCCAGATCATGTC	1228	CCCAGUAUGGUUCCAGAUCAUGUC	1551
Human-Exon 7	1	1	TTTA	TATTTGTCTTtgtgtatgtgtgta	1229	UAUUUGUCUUuguguaugugugua	1552
Human-Exon 7	2	1	TTTG	TCTTtgtgtatgtgtgtatgtgta	1230	UCUUuguguaugugugguaugugua	1553
Human-Exon 7	3	1	TTtg	tgtatgtgtgtatgtgtatgtgtt	1231	uguaugugugguaugugguauguguu	1554
Human-Exon 7	4	1	ttTT	AGGCCAGACCTATTTGACTGGAAT	1232	AGGCCAGACCUAUUUGACUGGAAU	1555
Human-Exon 7	5	1	tTTA	GGCCAGACCTATTTGACTGGAATA	1233	GGCCAGACCUAUUUGACUGGAAUA	1556
Human-Exon 7	6	1	TTTG	ACTGGAATAGTGTGGTTTGCCAGC	1234	ACUGGAAUAGUGUGGUUUGCCAGC	1557

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Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 7	7	1	TTTG	CCAGCAGTCAGCCACACAACGACT	1235	CCAGCAGUCAGCCACACAACGACU	1558
Human-Exon 7	8	-1	TTTC	TCTATGCCTAATTGATATCTGGCG	1236	UCUAUGCCUAAUUGAUAUCUGGCG	1559
Human-Exon 7	9	-1	TTTA	CCAACCTTCAGGATCGAGTAGTTT	1237	CCAACCUUCAGGAUCGAGUAGUUU	1560
Human-Exon 7	10	1	TTTC	TGGACTACCACTGCTTTTAGTATG	1238	UGGACUACCACUGCUUUUAGUAUG	1561
Human-Exon 7	11	1	TTTT	AGTATGGTAGAGTTTAATGTTTTC	1239	AGUAUGGUAGAGUUUAAUGUUUUC	1562
Human-Exon 7	12	1	TTTA	GTATGGTAGAGTTTAATGTTTTCA	1240	GUAUGGUAGAGUUUAAUGUUUUCA	1563
Human-Exon 8	1	-1	TTTG	AGACTCTAAAAGGATAATGAACAA	1241	AGACUCUAAAAGGAUAAUGAACAA	1564
Human-Exon 8	2	1	TTTA	ACTTTGATTTGTTCATTATCCTTT	1242	ACUUUGAUUUGUUCAUUAUCCUUU	1565
Human-Exon 8	3	-1	TTTC	TATATTTGAGACTCTAAAAGGATA	1243	UAUAUUUGAGACUCUAAAAGGAUA	1566
Human-Exon 8	4	1	TTTG	ATTTGTTCATTATCCTTTTAGAGT	1244	AUUUGUUCAUUAUCCUUUUAGAGU	1567
Human-Exon 8	5	-1	TTTG	GTTTCTATATTTGAGACTCTAAAA	1245	GUUUCUAUAUUUGAGACUCUAAAA	1568
Human-Exon 8	6	-1	TTTT	GGTTTCTATATTTGAGACTCTAAA	1246	GGUUUCUAUAUUUGAGACUCUAAA	1569
Human-Exon 8	7	-1	TTTT	TGGTTTCTATATTTGAGACTCTAA	1247	UGGUUUCUAUAUUUGAGACUCUAA	1570
Human-Exon 8	8	1	TTTG	TTCATTATCCTTTTAGAGTCTCAA	1248	UUCAUUAUCCUUUUAGAGUCUCAA	1571
Human-Exon 8	9	1	TTTT	AGAGTCTCAAATATAGAAACCAAA	1249	AGAGUCUCAAAUAUAGAAACCAAA	1572
Human-Exon 8	10	1	TTTA	GAGTCTCAAATATAGAAACCAAAA	1250	GAGUCUCAAAUAUAGAAACCAAAA	1573
Human-Exon 8	11	-1	TTTC	CACTTCCTGGATGGCTTCAATGCT	1251	CACUUCCUGGAUGGCUUCAAUGCU	1574

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 8	12	1	TTTT	GCCTCAACAAGTGAGCATTGAAGC	1252	GCCUCAACAAGUGAGCAUUGAAGC	1575
Human-Exon 8	13	1	TTTG	CCTCAACAAGTGAGCATTGAAGCC	1253	CCUCAACAAGUGAGCAUUGAAGCC	1576
Human-Exon 8	14	-1	TTTA	GGTGGCCTTGGCAACATTTCCACT	1254	GGUGGCCUUGGCAACAUUUCCACU	1577
Human-Exon 8	15	-1	TTTA	GTCACTTTAGGTGGCCTTGGCAAC	1255	GUCACUUUAGGUGGCCUUGGCAAC	1578
Human-Exon 8	16	-1	TTTG	ATGATGTAACTGAAAATGTTCTTC	1256	AUGAUGUAACUGAAAAUGUUCUUC	1579
Human-Exon 8	17	-1	TTTA	CCTGTTGAGAATAGTGCATTTGAT	1257	CCUGUUGAGAAUAGUGCAUUUGAU	1580
Human-Exon 8	18	1	TTTT	CAGTTACATCATCAAATGCACTAT	1258	CAGUUACAUCAUCAAAUGCACUAU	1581
Human-Exon 8	19	1	TTTC	AGTTACATCATCAAATGCACTATT	1259	AGUUACAUCAUCAAAUGCACUAUU	1582
Human-Exon 8	20	-1	TTTA	CACACTTTACCTGTTGAGAATAGT	1260	CACACUUUACCUGUUGAGAAUAGU	1583
Human-Exon 8	21	1	TTTT	CTGTTTTATATGCATTTTTAGGTA	1261	CUGUUUUAUAUGCAUUUUUAGGUA	1584
Human-Exon 8	22	1	TTTC	TGTTTTATATGCATTTTTAGGTAT	1262	UGUUUUAUAUGCAUUUUUAGGUAU	1585
Human-Exon 8	23	1	TTTT	ATATGCATTTTTAGGTATTACGTG	1263	AUAUGCAUUUUUAGGUAUUACGUG	1586
Human-Exon 8	24	1	TTTA	TATGCATTTTTAGGTATTACGTGC	1264	UAUGCAUUUUUAGGUAUUACGUGC	1587
Human-Exon 8	25	1	TTTT	TAGGTATTACGTGCACatatatat	1265	UAGGUAUUACGUGCACauauauau	1588
Human-Exon 8	26	1	TTTT	AGGTATTACGTGCACatatatata	1266	AGGUAUUACGUGCACauauauaua	1589
Human-Exon 8	27	1	TTTA	GGTATTACGTGCACatatatatat	1267	GGUAUUACGUGCACauauauauau	1590
Human-Exon 55	1	-1	TTTA	AGCAACAACTATAATATTGTGCAG	1268	AGCAACAACUAUAAUAUUGUGCAG	1591

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 55	2	1	TTTA	GTTCCTCCATCTTTCTCTTTTTAT	1269	GUUCCUCCAUCUUUCUCUUUUUAU	1592
Human-Exon 55	3	1	TTTC	TCTTTTTATGGAGTTCACTAGGTG	1270	UCUUUUUAUGGAGUUCACUAGGUG	1593
Human-Exon 55	4	1	TTTT	TATGGAGTTCACTAGGTGCACCAT	1271	UAUGGAGUUCACUAGGUGCACCAU	1594
Human-Exon 55	5	1	TTTT	ATGGAGTTCACTAGGTGCACCATT	1272	AUGGAGUUCACUAGGUGCACCAUU	1595
Human-Exon 55	6	1	TTTA	TGGAGTTCACTAGGTGCACCATTC	1273	UGGAGUUCACUAGGUGCACCAUUC	1596
Human-Exon 55	7	1	TTTA	ATAATTGCATCTGAACATTTGGTC	1274	AUAAUUGCAUCUGAACAUUUGGUC	1597
Human-Exon 55	8	1	TTTG	GTCCTTTGCAGGGTGAGTGAGCGA	1275	GUCCUUUGCAGGGUGAGUGAGCGA	1598
Human-Exon 55	9	-1	TTTC	TTCCAAAGCAGCCTCTCGCTCACT	1276	UUCCAAAGCAGCCUCUCGCUCACU	1599
Human-Exon 55	10	1	TTTG	CAGGGTGAGTGAGCGAGAGGCTGC	1277	CAGGGUGAGUGAGCGAGAGGCUGC	1600
Human-Exon 55	11	1	TTTG	GAAGAAACTCATAGATTACTGCAA	1278	GAAGAAACUCAUAGAUUACUGCAA	1601
Human-Exon 55	12	-1	TTTC	CAGGTCCAGGGGGAACTGTTGCAG	1279	CAGGUCCAGGGGGAACUGUUGCAG	1602
Human-Exon 55	13	-1	TTTT	CCAGGTCCAGGGGGAACTGTTGCA	1280	CCAGGUCCAGGGGGAACUGUUGCA	1603
Human-Exon 55	14	-1	TTTC	AGCTTCTGTAAGCCAGGCAAGAAA	1281	AGCUUCUGUAAGCCAGGCAAGAAA	1604
Human-Exon 55	15	1	TTTC	TTGCCTGGCTTACAGAAGCTGAAA	1282	UUGCCUGGCUUACAGAAGCUGAAA	1605
Human-Exon 55	16	-1	TTTC	CTTACGGGTAGCATCCTGTAGGAC	1283	CUUACGGGUAGCAUCCUGUAGGAC	1606
Human-Exon 55	17	-1	TTTA	CTCCCTTGGAGTCTTCTAGGAGCC	1284	CUCCCUUGGAGUCUUCUAGGAGCC	1607
Human-Exon 55	18	-1	TTTT	ACTCCCTTGGAGTCTTCTAGGAGC	1285	ACUCCCUUGGAGUCUUCUAGGAGC	1608

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
Human-Exon 55	19	-1	TTTC	ATCAGCTCTTTTACTCCCTTGGAG	1286	AUCAGCUCUUUUACUCCCUUGGAG	1609
Human-Exon 55	20	1	TTTC	CGCTTTAGCACTCTTGTGGATCCA	1287	CGCUUUAGCACUCUUGUGGAUCCA	1610
Human-Exon 55	21	1	TTTA	GCACTCTTGTGGATCCAATTGAAC	1288	GCACUCUUGUGGAUCCAAUUGAAC	1611
Human-Exon 55	22	-1	TTTG	TCCCTGGCTTGTCAGTTACAAGTA	1289	UCCCUGGCUUGUCAGUUACAAGUA	1612
Human-Exon 55	23	-1	TTTT	GTCCCTGGCTTGTCAGTTACAAGT	1290	GUCCCUGGCUUGUCAGUUACAAGU	1613
Human-Exon 55	24	-1	TTTG	TTTTGTCCCTGGCTTGTCAGTTAC	1291	UUUUGUCCCUGGCUUGUCAGUUAC	1614
Human-Exon 55	25	-1	TTTT	GTTTTGTCCCTGGCTTGTCAGTTA	1292	GUUUUGUCCCUGGCUUGUCAGUUA	1615
Human-Exon 55	26	1	TTTG	TACTTGTAACTGACAAGCCAGGGA	1293	UACUUGUAACUGACAAGCCAGGGA	1616
Human-G1-exon51		1	TTTA	gCTCCTACTCAGACTGTTACTCTG	1294	gCUCCUACUCAGACUGUUACUCUG	1617
Human-G2-exon51		1	TTTC	taccatgtattgctaaacaaagta	1295	uaccauguauugcuaaacaaagua	1618
Human-G3-exon51		-1	TTTA	attgaagagtaacaatttgagcca	1296	auugaagaguaacaauuugagcca	1619
mouse-Exon23-G1		1	TTTG	aggctctgcaaagttctTTGAAAG	1297	aggcucugcaaaguucuUUGAAAG	1620
mouse-Exon23-G2		1	TTTG	AAAGAGCAACAAAATGGCttcaac	1298	AAAGAGCAACAAAAUGGCuucaac	1621
mouse-Exon23-G3		1	TTTG	AAAGAGCAATAAAATGGCttcaac	1299	AAAGAGCAAUAAAAUGGCuucaac	1622
mouse-Exon23-G4		-1	TTTC	AAAGAACTTTGCAGAGCctcaaaa	1300	AAAGAACUUUGCAGAGCcucaaaa	1623

Targeted gRNA Exon	Guide #	Strand	PAM	DNA sequence*	SEQ ID NO.	RNA sequence*	SEQ ID NO.
mouse-Exon23-G5		-1	TTTA	ctgaatatctatgcattaataact	1301	cugaauaucuaugcauuaauaacu	1624
mouse-Exon23-G6		-1	TTTC	tattatattacagggcatattata	1302	uauuauauuacagggcauauuaua	1625
mouse-Exon23-G7		1	TTTC	Aggtaagccgaggtttggccttta	1303	Agguaagccgagguuuggccuuua	1626
mouse-Exon23-G8		1	TTTA	cccagagtccttcaaagatattga	1304	cccagaguccuucaaagauauuga	1627

* In this table, upper case letters represent sgRNA nucleotides that align to the exon sequence of the gene. Lower case letters represent sgRNA nucleotides that align to the intron sequence of the gene

Table 19 – Additional gRNA targeting sequences

Name	Species	Gene	Target	Strand	Sequence	SEQ ID NO	PAM
DCR1	Human	DMD	Intron 50	+	attggctttgatttcccta	1628	GGG
DCR2	Human	DMD	Intron 50	−	tgtagagtaagtcagccta	1629	TGG
DCR3	Human	DMD	Exon 51-55′	+	cctactcagactgttactc	1630	TGG
DCR4	Human	DMD	Exon 51-53′	+	ttggacagaacttaccgac	1631	TGG
DCR5	Human	DMD	Intron 51	−	cagttgcctaagaactggt	1632	GGG
DCR6	Human	DMD	Intron 44	−	GGGCTCCACCCTCACGAGT	1633	GGG
DCR7	Human	DMD	Intron 55	+	TTTGCTTCGCTATAAAACG	1634	AGG
DCR8	Human	DMD	Exon 41	+	TCTGAGGATGGGGCCGCAA	1635	TGG
DCR9	Human	DMD	Exon 44	−	GATCTGTCAAATCGCCTGC	1636	AGG
DCR10	Human	DMD	Exon 45	+	CCAGGATGGCATTGGGCAG	1637	CGG
DCR11	Human	DMD	Exon 45	+	CTGAATCTGCGGTGGCAGG	1638	AGG
DCR12	Human	DMD	Exon 46	−	TTCTTTTGTTCTTCTAGCc	1639	TGG
DCR13	Human	DMD	Exon 46	+	GAAAAGCTTGAGCAAGTCA	1640	AGG
DCR14	Human	DMD	Exon 47	+	GAAGAGTTGCCCCTGCGCC	1641	AGG
DCR15	Human	DMD	Exon 47	+	ACAAATCTCCAGTGGATAA	1642	AGG
DCR16	Human	DMD	Exon 48	−	TGTTTCTCAGGTAAAGCTC	1643	TGG
DCR17	Human	DMD	Exon 48	+	GAAGGACCATTTGACGTTa	1644	AGG
DCR18	Human	DMD	Exon 49	−	AACTGCTATTTCAGTTTCc	1645	TGG
DCR19	Human	DMD	Exon 49	+	CCAGCCACTCAGCCAGTGA	1646	AGG
DCR20	Human	DMD	Exon 50	+	gtatgcttttctgttaaag	1647	AGG
DCR21	Human	DMD	Exon 50	+	CTCCTGGACTGACCACTAT	1648	TGG
DCR22	Human	DMD	Exon 52	+	GAACAGAGGCGTCCCCAGT	1649	TGG
DCR23	Human	DMD	Exon 52	+	GAGGCTAGAACAATCATTA	1650	CGG
DCR24	Human	DMD	Exon 53	+	ACAAGAACACCTTCAGAAC	1651	CGG
DCR25	Human	DMD	Exon 53	−	GGTTTCTGTGATTTTCTTT	1652	TGG
DCR26	Human	DMD	Exon 54	+	GGCCAAAGACCTCCGCCAG	1653	TGG
DCR27	Human	DMD	Exon 54	+	TTGGAGAAGCATTCATAAA	1654	AGG

DCR28	Human	DMD	Exon 55	−	TCGCTCACTCACCctgcaa	1655	AGG
DCR29	Human	DMD	Exon 55	+	AAAAGAGCTGATGAAACAA	1656	TGG
DCR30	Human	DMD	5′UTR/ Exon 1	+	TAcACTTTTCaAAATGCTT	1657	TGG
DCR31	Human	DMD	Exon 51	+	gagatgatcatcaagcaga	1658	AGG
DCR32	Mouse	DMD	mdx	+	ctttgaaagagcaaTaaaa	1659	TGG
DCR33	Human	DMD	Intron 44	−	CACAAAAGTCAAATCGGAA	1660	TGG
DCR34	Human	DMD	Intron 44	−	ATTTCAATATAAGATTCGG	1661	AGG
DCR35	Human	DMD	Intron 55	−	CTTAAGCAATCCCGAACTC	1662	TGG
DCR36	Human	DMD	Intron 55	−	CCTTCTTTATCCCCTATCG	1663	AGG
DCR40	Mouse	DMD	Exon 23	−	aggccaaacctcggcttac	1664	NNGRR
DCR41	Mouse	DMD	Exon 23	+	TTCGAAAATTTCAGgtaag	1665	NNGRR
DCR42	Mouse	DMD	Exon 23	+	gcagaacaggagataacag	1666	NNGRRT
DCR43	Mouse	ACVR2B	Exon 1	+	gcggccctcgcccttctct	1667	ggggat
DCR48	Human	DMD	Intron 45	−	TAGTGATCGTGGATACGAG	1668	AGG
DCR49	Human	DMD	Intron 45	−	TACAGCCCTCGGTGTATAT	1669	TGG
DCR50	Human	DMD	Intron 52	−	GGAAGGAATTAAGCCCGAA	1670	TGG
DCR51	Human	DMD	Intron 53	−	GGAACAGCTTTCGTAGTTG	1671	AGG
DCR52	Human	DMD	Intron 54	+	ATAAAGTCCAGTGTCGATC	1672	AGG
DCR53			Intron 54	+	AAAACCAGAGCTTCGGTCA	1673	AGG
DCR54	Mouse	Rosa26	ZFN region	+	GAGTCTTCTGGGCAGGCTTAA	1674	TGG
DCR55	Mouse	Rosa26	mRNA	−	TCGGGTGAGCATGTCTTTAAT	1675	TGG
DCR49	Human	DMD	Ex 51	−	gtgtcaccagagtaacagt	1676	ctgagt
DCR50	Human	DMD	Ex 51	+	tgatcatcaagcagaaggt	1677	atgag
DCR60	Mouse	DMD	Exon 23	+	AACTTCGAAAATTTCAGgta	1678	agccgagg
DCR61	Mouse	DMD	Intron 22	+	gaaactcatcaaatatgcgt	1679	gttagtgt
DCR62	Mouse	DMD	Intron 22	−	tcatttacactaacacgcat	1680	atttgatg
DCR63	Mouse	DMD	Intron 22	+	gaatgaaactcatcaaatat	1681	gcgtgtta
DCR64	Mouse	DMD	Intron 23	−	tcatcaatatctttgaagga	1682	ctctgggt
DCR65	Mouse	DMD	Intron 23	−	tgttttcataggaaaaatag	1683	gcaagttg

DCR66	Mouse	DMD	Intron 23	+	aattggaaaatgtgatggga	1684	aacagata
DCR67	Human	DMD	Exon 51	+	atgatcatcaagcagaaggt	1685	atgagaaa
DCR68	Human	DMD	Exon 51	+	agatgatcatcaagcagaag	1686	gtatgaga
DCR69	Human	DMD	Exon 51	−	catttttctcataccttct	1687	gcttgatg
DCR70	Human	DMD	Exon 51	+	tcctactcagactgttactc	1688	tggtgaca
DCR71	Human	DMD	Exon 51	−	acaggttgtgtcaccagagt	1689	aacagtct
DCR72	Human	DMD	Exon 51	−	ttatcatttttctcatacc	1690	ttctgctt
DCR73	Human	DMD	Intron 51	−	ttgcctaagaactggtggga	1691	aatggtct
DCR74	Human	DMD	Intron 51	−	aaacagttgcctaagaactg	1692	gtgggaaa
DCR75	Human	DMD	Intron 51	+	tttcccaccagttcttaggc	1693	aactgttt
DCR76	Human	DMD	Intron 50	+	tggctttgatttccctaggg	1694	tccagctt
DCR77	Human	DMD	Intron 50	−	tagggaaatcaaagccaatg	1695	aaacgttc
DCR78	Human	DMD	Intron 50	−	gaccctagggaaatcaaagc	1696	caatgaaa
DCR79	Human	DMD	Intron 44	−	TGAGGGCTCCACCCTCACGA	1697	GTGGGTTT
DCR80	Human	DMD	Intron 44	−	AAGGATTGAGGGCTCCACCC	1698	TCACGAGT
DCR81	Human	DMD	Intron 44	−	GCTCCACCCTCACGAGTGGG	1699	TTTGGTTC
DCR82	Human	DMD	Intron 55	−	TATCCCCTATCGAGGAAACC	1700	ACGAGTTT
DCR83	Human	DMD	Intron 55	+	GATAAAGAAGGCCTATTTCA	1701	TAGAGTTG
DCR84	Human	DMD	Intron 55	−	AGGCCTTCTTTATCCCCTAT	1702	CGAGGAAA
DCR85	Human	DMD	Intron 44	−	TGAGGGCTCCACCCTCACGA	1703	GTGGGT
DCR86	Human	DMD	Intron 55	+	GATAAAGAAGGCCTATTTCA	1704	TAGAGT
DCR1	Human	DMD	Intron 50	+	attggctttgatttcccta	1705	GGG
DCR2	Human	DMD	Intron 50	−	tgtagagtaagtcagccta	1706	TGG
DCR3	Human	DMD	Exon 51-5′	+	cctactcagactgttactc	1707	TGG
DCR4	Human	DMD	Exon 51-3′	+	ttggacagaacttaccgac	1708	TGG
DCR5	Human	DMD	Intron 51	−	cagttgcctaagaactggt	1709	GGG
DCR6	Human	DMD	Intron 44	−	GGGCTCCACCCTCACGAGT	1710	GGG
DCR7	Human	DMD	Intron 55	+	TTTGCTTCGCTATAAAACG	1711	AGG
DCR8	Human	DMD	Exon 41	+	TCTGAGGATGGGGCCGCAA	1712	TGG
DCR9	Human	DMD	Exon 44	−	GATCTGTCAAATCGCCTGC	1713	AGG

DCR10	Human	DMD	Exon 45	+	CCAGGATGGCATTGGGCAG	1714	CGG
DCR11	Human	DMD	Exon 45	+	CTGAATCTGCGGTGGCAGG	1715	AGG
DCR12	Human	DMD	Exon 46	−	TTCTTTTGTTCTTCTAGCc	1716	TGG
DCR13	Human	DMD	Exon 46	+	GAAAAGCTTGAGCAAGTCA	1717	AGG
DCR14	Human	DMD	Exon 47	+	GAAGAGTTGCCCCTGCGCC	1718	AGG
DCR15	Human	DMD	Exon 47	+	ACAAATCTCCAGTGGATAA	1719	AGG
DCR16	Human	DMD	Exon 48	−	TGTTTCTCAGGTAAAGCTC	1720	TGG
DCR17	Human	DMD	Exon 48	+	GAAGGACCATTTGACGTTa	1721	AGG
DCR18	Human	DMD	Exon 49	−	AACTGCTATTTCAGTTTCc	1722	TGG
DCR19	Human	DMD	Exon 49	+	CCAGCCACTCAGCCAGTGA	1723	AGG
DCR20	Human	DMD	Exon 50	+	gtatgcttttctgttaaag	1724	AGG
DCR21	Human	DMD	Exon 50	+	CTCCTGGACTGACCACTAT	1725	TGG
DCR22	Human	DMD	Exon 52	+	GAACAGAGGCGTCCCCAGT	1726	TGG
DCR23	Human	DMD	Exon 52	+	GAGGCTAGAACAATCATTA	1727	CGG
DCR24	Human	DMD	Exon 53	+	ACAAGAACACCTTCAGAAC	1728	CGG
DCR25	Human	DMD	Exon 53	−	GGTTTCTGTGATTTTCTTT	1729	TGG
DCR26	Human	DMD	Exon 54	+	GGCCAAAGACCTCCGCCAG	1730	TGG
DCR27	Human	DMD	Exon 54	+	TTGGAGAAGCATTCATAAA	1731	AGG
DCR28	Human	DMD	Exon 55	−	TCGCTCACTCACCctgcaa	1732	AGG
DCR29	Human	DMD	Exon 55	+	AAAAGAGCTGATGAAACAA	1733	TGG
DCR30	Human	DMD	5′UTR/ Exon 1	+	TAcACTTTTCaAAATGCTT	1734	TGG
DCR31	Human	DMD	Exon 51	+	gagatgatcatcaagcaga	1735	AGG
DCR32	Mouse	DMD	mdx	+	ctttgaaagagcaaTaaaa	1736	TGG
DCR33	Human	DMD	Intron 44	−	CACAAAAGTCAAATCGGAA	1737	TGG
DCR34	Human	DMD	Intron 44	−	ATTTCAATATAAGATTCGG	1738	AGG
DCR35	Human	DMD	Intron 55	−	CTTAAGCAATCCCGAACTC	1739	TGG
DCR36	Human	DMD	Intron 55	−	CCTTCTTTATCCCCTATCG	1740	AGG
DCR40	Mouse	DMD	Exon 23	−	aggccaaacctcggcttac	1741	NNGRR
DCR41	Mouse	DMD	Exon 23	+	TTCGAAAATTTCAGgtaag	1742	NNGRR

DCR42	Mouse	DMD	Exon 23	+	gcagaacaggagataacag	1743	NNGRRT
DCR43	Mouse	ACVR2B	Exon 1	+	gcggccctcgcccttctct	1744	ggggat
DCR48	Human	DMD	Intron 45	−	TAGTGATCGTGGATACGAG	1745	AGG
DCR49	Human	DMD	Intron 45	−	TACAGCCCTCGGTGTATAT	1746	TGG
DCR50	Human	DMD	Intron 52	−	GGAAGGAATTAAGCCCGAA	1747	TGG
DCR51	Human	DMD	Intron 53	−	GGAACAGCTTTCGTAGTTG	1748	AGG
DCR52	Human	DMD	Intron 54	+	ATAAAGTCCAGTGTCGATC	1749	AGG
DCR53			Intron 54	+	AAAACCAGAGCTTCGGTCA	1750	AGG
DCR54	Mouse	Rosa26	ZFN region	+	GAGTCTTCTGGGCAGGCTTAA	1751	TGG
DCR55	Mouse	Rosa26	mRNA	−	TCGGGTGAGCATGTCTTTAAT	1752	TGG
DCR49	Human	DMD	Ex 51	−	gtgtcaccagagtaacagt	1753	ctgagt
DCR50	Human	DMD	Ex 51	+	tgatcatcaagcagaaggt	1754	atgag
DCR60	Mouse	DMD	Exon 23	+	AACTTCGAAAATTTCAGgta	1755	agccgagg
DCR61	Mouse	DMD	Intron 22	+	gaaactcatcaaatatgcgt	1756	gttagtgt
DCR62	Mouse	DMD	Intron 22	−	tcatttacactaacacgcat	1757	atttgatg
DCR63	Mouse	DMD	Intron 22	+	gaatgaaactcatcaaatat	1758	gcgtgtta
DCR64	Mouse	DMD	Intron 23	−	tcatcaatatctttgaagga	1759	ctctgggt
DCR65	Mouse	DMD	Intron 23	−	tgttttcataggaaaaatag	1760	gcaagttg
DCR66	Mouse	DMD	Intron 23	+	aattggaaaatgtgatggga	1761	aacagata
DCR67	Human	DMD	Exon 51	+	atgatcatcaagcagaaggt	1762	atgagaaa
DCR68	Human	DMD	Exon 51	+	agatgatcatcaagcagaag	1763	gtatgaga
DCR69	Human	DMD	Exon 51	−	catttttctcataccttct	1764	gcttgatg
DCR70	Human	DMD	Exon 51	+	tcctactcagactgttactc	1765	tggtgaca
DCR71	Human	DMD	Exon 51	−	acaggttgtgtcaccagagt	1766	aacagtct
DCR72	Human	DMD	Exon 51	−	ttatcatttttctcatacc	1767	ttctgctt
DCR73	Human	DMD	Intron 51	−	ttgcctaagaactggtggga	1768	aatggtct
DCR74	Human	DMD	Intron 51	−	aaacagttgcctaagaactg	1769	gtgggaaa
DCR75	Human	DMD	Intron 51	+	tttcccaccagttcttaggc	1770	aactgttt
DCR76	Human	DMD	Intron 50	+	tggctttgatttccctaggg	1771	tccagctt

DCR77	Human	DMD	Intron 50	−	tagggaaatcaaagccaatg	1772	aaacgttc
DCR78	Human	DMD	Intron 50	−	gaccctagggaaatcaaagc	1773	caatgaaa
DCR79	Human	DMD	Intron 44	−	TGAGGGCTCCACCCTCACGA	1774	GTGGGTTT
DCR80	Human	DMD	Intron 44	−	AAGGATTGAGGGCTCCACCC	1775	TCACGAGT
DCR81	Human	DMD	Intron 44	−	GCTCCACCCTCACGAGTGGG	1776	TTTGGTTC
DCR82	Human	DMD	Intron 55	−	TATCCCCTATCGAGGAAACC	1777	ACGAGTTT
DCR83	Human	DMD	Intron 55	+	GATAAAGAAGGCCTATTTCA	1778	TAGAGTTG
DCR84	Human	DMD	Intron 55	−	AGGCCTTCTTTATCCCCTAT	1779	CGAGGAAA
DCR85	Human	DMD	Intron 44	−	TGAGGGCTCCACCCTCACGA	1780	GTGGGT
DCR86	Human	DMD	Intron 55	+	GATAAAGAAGGCCTATTTCA	1781	TAGAGT
		DMD			UAGAAGAUCUGAGCUCUGAG	1782	
		DMD			AGAUCUGAGCUCUGAGUGGA	1783	
		DMD			UCUGAGCUCUGAGUGGAAGG	1784	
		DMD			CCGUUUACUUCAAGAGCUGA	1785	
		DMD			AAGCAGCCUGACCUAGCUCC	1786	
		DMD			GCUCCUGGACUGACCACUAU	1787	
		DMD			CCCUCAGCUCUUGAAGUAAA	1788	
		DMD			GUCAGUCCAGGAGCUAGGUC	1789	
		DMD			UAGUGGUCAGUCCAGGAGCU	1790	
		DMD			GCUCCAAUAGUGGUCAGUCC	1791	
		DMD			UGGCCAAAGACCUCCGCCAG	1792	
		DMD			GUGGCAGACAAAUGUAGAUG	1793	
		DMD			UGUAGAUGUGGCAAAUGACU	1794	
		DMD			CUUGGCCCUGAAACUUCUCC	1795	
		DMD			CAGAGAAUAUCAAUGCCUCU	1796	
		DMD			CAGAGAAUAUCAAUGCCUCU	1797	
		DMD			CAUUUGUCUGCCACUGGCGG	1798	
		DMD			CUACAUUUGUCUGCCACUGG	1799	
		DMD			CAUCUACAUUUGUCUGCCAC	1800	
		DMD			AUAAUCCCGGAGAAGUUUCA	1801	
		DMD			UAUCAUCUGCAGAAUAAUCC	1802	
		DMD			UGUUAUCAUGUGGACUUUUC	1803	
		DMD			UGAUAUAUCAUUUCUCUGUG	1804	
		DMD			UUUAUGAAUGCUUCUCCAAG	1805	
		DMD			UUCUCCAGGCUAGAAGAACAA	1806	
		DMD			CUGCUCUUUUCCAGGUUCAAG	1807	

		DMD			GUCUGUUUCAGUUACUGGUGG	1808	
		DMD			UCCAGUUUCAUUUAAUUGUUU	1809	
		DMD			CUUAUGGGAGCACUUACAAGC	1810	
		DMD			UUGCUUCAUUACCUUCACUGG	1811	
		DMD			UUGUGUCACCAGAGUAACAGU	1812	
		DMD			AGUAACCACAGGUUGUGUCAC	1813	
		DMD			UUCAAAUUUUGGGCAGCGGUA	1814	
		DMD			CAAGAGGCUAGAACAAUCAUU	1815	
		DMD			UUGUACUUCAUCCCACUGAUU	1816	
		DMD			CUUCAGAACCGGAGGCAACAG	1817	
		DMD			CAACAGUUGAAUGAAAUGUUA	1818	
		DMD			GCCAAGCUUGAGUCAUGGAAG	1819	
		DMD			CUUGGUUUCUGUGAUUUUCUU	1820	
		DMD			UCAUUUCACAGGCCUUCAAGA	1821	
		DMD			CAGAAAUAUUCGUACAGUCUC	1822	
		DMD			CAAUUACCUCUGGGCUCCUGG	1823	
		DMD			GATACTAGGGTGGCAAATAG	1824	
		DMD			GTGTTCTTAAAAGAATGGTG	1825	
		DMD			GTCAAGAACAGCTGCAGAAC	1826	
		DMD			GCAGTTGAATGAAATGTTAA	1827	
		DMD			GATACTAGTGTGGCTCATAG	1828	
		DMD			GATACGATGGTGGCAAATCG	1829	
		DMD			GATACTAGGGTGGGGAATAA	1830	
		DMD			TTTTTCTTAAAAGAATGGTA	1831	
		DMD			TTGATCTTAGAAGAATGGTG	1832	
		DMD			GTTTTCTTGAAAAAATGGTG	1833	
		DMD			CTGTTCTTAAAAGGTTGGTG	1834	
		DMD			GAGTTCTTCAAAGAATAGTG	1835	
		DMD			TCTAGGGCAGCTGCAGAAC	1836	
		DMD			TCATTCACAGCTGCAGAAC	1837	
		DMD			CAAAGAATAGCTGCAGAAC	1838	
		DMD			TCAAGAACAGCTGCAGCAG	1839	
		DMD			TCAAGAACAGCTGCATCAC	1840	
		DMD			CAGTTACATGAAATGTTAA	1841	
		DMD			CATTTTAATGAAATGTTAA	1842	
		DMD			AAGTTGAATGAAATTTTAA	1843	
		DMD			CAGTGGAATAAAATGTTAA	1844	
		DMD			AAAGATATATAATGTCATGAAT	1845	
		DMD			GCAGAATCAAATATAATAGTCT	1846	
		DMD			AACAAATATCCCTTAGTATC	1847	

		DMD			AATGTATTTCTTCTATTCAA	1848	
		DMD			AACAATAAGTCAAATTTAATTG	1849	
		DMD			GAACTGGTGGGAAATGGTCTAG	1850	
		DMD			TCCTTTGGTAAATAAAAGTCCT	1851	
		DMD			TAGGAATCAAATGGACTTGGAT	1852	
		DMD			TAATTCTTTCTAGAAAGAGCCT	1853	
		DMD			CTCTTGCATCTTGCACATGTCC	1854	
		DMD			ACTTAGAGGTCTTCTACATACA	1855	
		DMD			TCAGAGGTGAGTGGTGAGGGGA	1856	
		DMD			ACACACAGCTGGGTTATCAGAG	1857	
		DMD			CACAGCTGGGTTATCAGAG	1858	
		DMD			ACACAGCTGGGTTATCAGAG	1859	
		DMD			CACACAGCTGGGTTATCAGAG	1860	
		DMD			AACACACAGCTGGGTTATCAGAG	1861	
		DMD			CTGSTGGGARATGGTCTAG	1862	
		DMD			ACTGGTGGGAAATGGTCTAG	1863	
		DMD			AACTGGTGGGAAATGGTCTAG	1864	
		DMD			AGAACTGGTGGGAAATGGTCTAG	1865	
		DMD			ATATCTTCTTAAATACCCGA	1866	
		DMD			AGTCTCACAAAACTGCAGAG	1867	
		DMD			TACTTATGTATTTTAAAAAC	1868	
		DMD			GAATAATTTCTATTATATTACA	1869	
		DMD			TTCGAAAATTTCAGGTAAGCCG	1870	
		DMD			TCATTTCTAAAAGTCTTTTGCC	1871	
		DMD			TTTGAGACACAGTATAGGTTAT	1872	
		DMD			ATATAATAGAAATTATTCAT	1873	
		DMD			TAATATGCCCTGTAATATAA	1874	
		DMD			TGATATCATCAATATCTTTG	1875	
		DMD			GCAATTAATTGGAAAATGTG	1876	
		DMD			CTTTAAGCTTAGGTAAAATCA	1877	
		DMD			CAGTAATGTGTCATACCTTC	1878	
		DMD			CAGGGCATATTATATTTAGA	1879	
		DMD			CAAAAGCCAAATCTATTTCA	1880	
		DMD			ATGCTTTGGTGGGAAGAAGTAGAGGA	1881	
		DMD			ATGCTTTGGTGGGAAGAATAGAGGAC	1882	
		DMD			TTGTGACAAGCTCACTAATTAGG	1883	
		DMD			AAGTTTGAAGAACTTTTACCAGG	1884	

		DMD			AGGCAGCGATAAAAAAAACCTGG	1885	
		DMD			GCTTTGGTGGGAAGAAGTAGAGG	1886	
		DMD			GCTGGGTGTCCCATTGAAA	1887	
		DMD			CAGCCGCTCGCTGCAGCAG	1888	
		DMD			TGGAGAGTTTGCAAGGAGC	1889	
		DMD			GTTTATTCAGCCGGGAGTC	1890	
		DMD			CGCCAGGAGGGGTGGGTCTA	1891	
		DMD			CCTTGGTGAGACTGGTAGA	1892	
		DMD			GTCTTCAGGTTCTGTTGCT	1893	
		DMD			ATATTCCTGATTTAAAAGT	1894	
		DMD			TTAAAAGTCGGCTGGTAGC	1895	
		DMD			CGGGCCGGGGGCGGGGTCC	1896	
		DMD			GCCCGAGCCGCGTGTGGAA	1897	
		DMD			CCTTCATTGCGGCGGGCTG	1898	
		DMD			CCGACCCCTCCCGGGTCCC	1899	
		DMD			CAGGACCGCGCTTCCCACG	1900	
		DMD			TGCACCCTGGGAGCGCGAG	1901	
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		DMD			GUGUUGUUUAACUGCUUCUU	2264	

		DMD			AACUGUCAGUUGCAUAUUCC	2265	
		DMD			CAGAAAGGAAUGCUGGUACC	2266	
		DMD			UCUGCCUACACAAUGAAUGG	2267	
		DMD			CACAGAUCAAUCCAAUUGUU	2268	
		DMD			UUGACAGGUGGAAAGUACAU	2269	
		DMD			ACAUUUUUAGGCUUGACAGG	2270	
		DMD			CUCUCCCAUGACAGACUCCC	2271	
		DMD			UUGGUAAGAGUUAUGAUAAG	2272	
		DMD			AACACAAAUUAAGUUCACCU	2273	
		DMD			AGGAUCAGUGCUGUAGUGCC	2274	
		DMD			GGCCGUUUAUUAUUAUUGAC	2275	
		DMD			UCUCAGGAUUGCUAUGCAAC	2276	
		DMD			CAGGAAGACAUACCAUGUAA	2277	
		DMD			AGCAGGGCUCUUUCAGUUUC	2278	
		DMD			UAACAUUUUCAGCUUGAACC	2279	
		DMD			UCAAGCUGAAAAUGUUACAC	2280	
		DMD			GUAACAUUUUCAGCUUGAAC	2281	
		DMD			CAGAAUGAAUUUUGGAGCAC	2282	
		DMD			UUUAUUAUUAUUGACUGGUG	2283	
		DMD			AGAAGAAUCUGACCUUUACA	2284	
		DMD			GCAGGGCUCUUUCAGUUUCU	2285	
		DMD			CUAAACAGUAGCCAGGCGUG	2286	
		DMD			CGCCUGGCUACUGUUUAGUG	2287	
		DMD			CUCCGCACUAAACAGUAGCC	2288	
		DMD			GUAGCCAGGCGUGUGGAUGU	2289	
		DMD			CUUGGCUUUGACUAUUCUGC	2290	
		DMD			AGUAGCCAGGCGUGUGGAUG	2291	
		DMD			UCCUCCCACAUCCACACGCC	2292	
		DMD			UUGGCUUUGACUAUUCUGCU	2293	
		DMD			AUAAUGUCUCUGGCUUGUAA	2294	
		DMD			UGGUACCCGGCAGCUCUCUG	2295	
		DMD			GUGGGAGGAACCUCAAAGAG	2296	
		DMD			UGACUAUUCUGCUGGGAACA	2297	
		DMD			CUCUCUGAGGAAUGUUCCCU	2298	
		DMD			AACAUUCCUCAGAGAGCUGC	2299	
		DMD			AUUCUGAAGCUCCAAACAAU	2300	
		DMD			UAAAUUACUCUGCUAAAGUA	2301	
		DMD			AGUACAAACCAGGUUUGUAC	2302	
		DMD			AUAUCCUUCCAGUACAAACC	2303	
		DMD			CAAACCAGGUUUGUACUGGA	2304	

		DMD			GGCAGCUAAAGCAUCACUGA	2305	
		DMD			AUCUCUGAGUAGUACAAACC	2306	
		DMD			GUGUCCCAUUCUCUUUGACU	2307	
		DMD			UGUGUCCCAUUCUCUUUGAC	2308	
		DMD			UUCUGAAUGUUGAACAAGUA	2309	
		DMD			GUCUCCCAGUCAAAGAGAAU	2310	
		DMD			AUUCUCUUUGACUGGGAGAC	2311	
		DMD			UCUUUGACUGGGAGACAGGC	2312	
		DMD			GUGGUGUCCUUUGAAUAUGC	2313	
		DMD			AGAUUGUCCAGGAUAUAAUU	2314	
		DMD			UUAGCAACCAAAUUAUAUCC	2315	
		DMD			GUUGAAAUUAAACUACACAC	2316	
		DMD			AUCUUUACCUGCAUAUUCAA	2317	
		DMD			GUGUCCUUUGAAUAUGC	2318	
		DMD			UUGUCCAGGAUAUAAUU	2319	
		DMD			GCAACCAAAUUAUAUCC	2320	
		DMD			GAAAUUAAACUACACAC	2321	
		DMD			UUUACCUGCAUAUUCAA	2322	
		DMD			UACACAUUUUUAGGCUUGAC	2323	
		DMD			CAUUCCUGGGAGUCUGUCAU	2324	
		DMD			UGUAUGAUGCUAUAAUACCA	2325	
		DMD			GUGGAAAGUACAUAGGACCU	2326	
		DMD			UCUUAUCAUAACUCUUACCA	2327	
		DMD			ACAUUUUUAGGCUUGAC	2328	
		DMD			UCCUGGGAGUCUGUCAU	2329	
		DMD			AUGAUGCUAUAAUACCA	2330	
		DMD			GAAAGUACAUAGGACCU	2331	
		DMD			UAUCAUAACUCUUACCA	2332	
		DMD			GAGTTCCTACTCAGACTGTTACTC	2333	
		DMD			GTGAGTTCCTACTCAGACTGTTACTC	2334	
		DMD			GTCTGAGTTCCTACTCAGACTGTTACTC	2335	
		DMD			AAAGATATATAATGTCATGAAT	2336	
		DMD			GCAGAATCAAATATAATAGTCT	2337	
		DMD			AACAAATATCCCTTAGTATC	2338	
		DMD			AATGTATTTCTTCTATTCAA	2339	
		DMD			AACAATAAGTCAAATTTAATTG	2340	
		DMD			GAACTGGTGGGAAATGGTCTAG	2341	
		DMD			TCCTTTGGTAAATAAAAGTCCT	2342	
		DMD			TAGGAATCAAATGGACTTGGAT	2343	

		DMD			TAATTCTTTCTAGAAAGAGCCT	2344	
		DMD			CTCTTGCATCTTGCACATGTCC	2345	
		DMD			ACTTAGAGGTCTTCTACATACA	2346	
		DMD			TCAGAGGTGAGTGGTGAGGGGA	2347	
		DMD			ACACACAGCTGGGTTATCAGAG	2348	
		DMD			CACAGCTGGGTTATCAGAG	2349	
		DMD			ACACAGCTGGGTTATCAGAG	2350	
		DMD			CACACAGCTGGGTTATCAGAG	2351	
		DMD			AACACACAGCTGGGTTATCAGAG	2352	
		DMD			CTGSTGGGARATGGTCTAG	2353	
		DMD			ACTGGTGGGAAATGGTCTAG	2354	
		DMD			AACTGGTGGGAAATGGTCTAG	2355	
		DMD			AGAACTGGTGGGAAATGGTCTAG	2356	
		DMD			ATATCTTCTTAAATACCCGA	2357	
		DMD			AGTCTCACAAAACTGCAGAG	2358	
		DMD			TACTTATGTATTTTAAAAAC	2359	
		DMD			GAATAATTTCTATTATATTACA	2360	
		DMD			TTCGAAAATTTCAGGTAAGCCG	2361	
		DMD			TCATTTCTAAAAGTCTTTTGCC	2362	
		DMD			TTTGAGACACAGTATAGGTTAT	2363	
		DMD			ATATAATAGAAATTATTCAT	2364	
		DMD			TAATATGCCCTGTAATATAA	2365	
		DMD			TGATATCATCAATATCTTTG	2366	
		DMD			GCAATTAATTGGAAAATGTG	2367	
		DMD			CTTTAAGCTTAGGTAAAATCA	2368	
		DMD			CAGTAATGTGTCATACCTTC	2369	
		DMD			CAGGGCATATTATATTTAGA	2370	
		DMD			CAAAAGCCAAATCTATTTCA	2371	
		DMD			ATGCTTTGGTGGGAAGAAGTAGAGGA	2372	
		DMD			ATGCTTTGGTGGGAAGAATAGAGGAC	2373	
		DMD			TTGTGACAAGCTCACTAATTAGG	2374	
		DMD			AAGTTTGAAGAACTTTTACCAGG	2375	
		DMD			AGGCAGCGATAAAAAAAACCTGG	2376	
		DMD			GCTTTGGTGGGAAGAAGTAGAGG	2377	

VII. Examples

The following examples are included to demonstrate preferred embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the disclosure, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

EXAMPLE 1

Genomic editing with CRISPR/Cas9 is a promising new approach for correcting or mitigating disease-causing mutations. Duchenne muscular dystrophy (DMD) is associated with lethal degeneration of cardiac and skeletal muscle caused by more than 3000 different mutations in the X-linked dystrophin gene (DMD). Most of these mutations are clustered in "hotspots." There is a fortuitous correspondence between the eukaryotic splice acceptor and splice donor sequences and the protospacer adjacent motif sequences that govern prokaryotic CRISPR/Cas9 target gene recognition and cleavage. Taking advantage of this correspondence, optimal guide RNAs capable of introducing insertion/deletion (indel) mutations by nonhomologous end joining that abolish conserved RNA splice sites in 12 exons that potentially allow skipping of the most common mutant or out-of-frame DMD exons within or nearby mutational hotspots were screened. Correction of DMD mutations by exon skipping is referred to herein as "myoediting." In proof-of-concept studies, myoediting was performed in representative induced pluripotent stem cells from multiple patients with large deletions, point mutations, or duplications within the DMD gene and efficiently restored dystrophin protein expression in derivative cardiomyocytes. In three-dimensional engineered heart muscle (EHM), myoediting of DMD mutations restored dystrophin expression and the corresponding mechanical force of contraction. Correcting only a subset of cardiomyocytes (30 to 50%) was sufficient to rescue the mutant EHM phenotype to near-normal control levels. Thus, abolishing conserved RNA splicing acceptor/donor sites and directing the splicing machinery to skip mutant or out-of-frame exons through myoediting allow correction of the cardiac abnormalities associated with DMD by eliminating the underlying genetic basis of the disease.

Identification of optimal guide RNAs to target 12 different exons associated with hotspot regions of DMD mutations

A list of the top 12 exons that, when skipped, can potentially restore the dystrophin open reading frame in most of the hotspot regions of DMD mutations is shown in Table 5. As an initial step toward correcting a majority of human DMD mutations by exon skipping, pools of guide RNAs were screened to target the top 12 exons of the human DMD gene (Fig. 1A and 1B). Three to six PAM sequences (NAG or NGG) were selected to target the 3′ or 5′ splice sites, respectively, of each exon (Fig. 1A and Table 5). These guide RNAs were cloned in plasmid SpCas9-2A-GFP. Indels that remove essential splice donor or acceptor sequences allow for skipping of the corresponding target exon. On the basis of the frequency of known DMD mutations, these guide RNAs would be predicted to be capable of rescuing dystrophin function in up to 60% of DMD patients.

To test the feasibility and efficacy of this strategy in the human genome, human embryonic kidney 293 cells (239 cells) were used to target the splice acceptor site of exon 51 (FIG. 1C). Transfected 293 cells were sorted by green fluorescent protein (GFP) expression, and gene editing efficiency was detected by the mismatch-specific T7E1 endo-nuclease assay (FIG. 6A). The ability of three guide RNAs (Ex51-g1, Ex51-g2, and Ex51-g3) to target the splice acceptor site of exon 51 is shown in Table 5 and Fig. 2B. In GFP-positive sorted 293 cells, Ex51-g3 showed high editing activity, whereas Ex51-g1 and Ex51-g2 had no detectable activity. Next, cleavage efficiency of guide RNAs, which target the top 12 exons, including exons 51, 45, 53, 44, 46, 52, 50, 43, 6, 7, 8, and 55, was evaluated. One or two guide RNAs with the highest efficiency of editing of each exon are shown in Fig. 1C. The selected guide RNAs for exons 51, 45, and 55 use NAG as the PAM (Table 5). Genomic polymerase chain reaction (PCR) products from the myoedited top 12 exons were cloned and sequenced (Fig. 5A and Table 20). Indels were observed that removed essential splice sites or shifted the open reading frame (Fig. 5A). In brain and kidney tissues, an N-terminally truncated form of dystrophin (Dp140) is transcribed from an alternative promoter in intron 44. Skipping of six targeted exons (exons 51, 53, 46, 52, 50, and 55) in Dp140 mRNA was confirmed in 293 cells by sequencing of reverse transcription PCR (RT-PCR) products (Fig. 5B).

Table 20: Sequence of primers for top 12 exons.

PCR/T7E1 and RT-PCR primers					
Exon #	**PCR/T7E1**	**SEQ ID NO:**	**RT-PCR**		**SEQ ID NO:**
51	F: TTCCCTGGCAAGGTCTGA	2427	F-E47:	CCCAGAAGAGCAAGATAAACTTGAA	2451
	R: ATCCTCAAGGTCACCCACC	2428	R-E52:	CTCTGTTCCAAATCCTGCATTGT	2452
45	F: GTCTTTCTGTCTTGTATCCTTTGG	2429			
	R: AATGTTAGTGCCTTTCACCC	2430			
53	F: GGGAAATCAGGCTGATGGGT	2431	F-E52:	CAAGACCAGCAATCAAGAGGCTAG	2453
	R: GTCTACTGTTCATTTCAGC	2432	R-E54:	TCATGTGGACTTTTCTGGTATCATC	2454
44	F: GCAGGAAACTATCAGAGTG	2433			
	R: ACACCTTGCTGTTACGAT	2434			
46	F: CCACCAAACCTGGCAAAT	2435	F-E45:	GAACTCCAGGATGGCATTGG	2455
	R: GAACTATGAATAACCTAATGGGCAG	2436	R-E52:	CTCTGTTCCAAATCCTGCATTGT	2456
52	F: TTCTTACTCAAGGCATTCAGAC	2437	F-E51:	GAAACTGCCATCTCCAAACTAGAAA	2457
	R: GGTCACCACACCCATCAAT	2438	R-E54:	TTCTCCAAGAGGCATTGATATTCTC	2458
50	F: TGCCTGGAGAAAGGGTTT	2439	R-E47:	CCCAGAAGAGCAAGATAAACTTGAA	2459
	R: GCACAGTCAATAACACAAAGGT	2440	R-E52:	CTCTGTTCCAAATCCTGCATTGT	2460
43	F: AGCGATCCACTCTCTCAGGATG	2441			
	R: GCACCTCAATGCCCCAATCTGATTTACG	2442			
6	F: GGGTCTAATATGGCAGAATCCA	2443			
	R: GTTGTAAAGTAGGACATGATCTGG	2444			
7	F: AGGACTATGGGCATTGGTT	2445			
	R: GTGTAGAAATGACAAGTCTCAGATG	2446			
8	F: GAAAGCTACTCTGTTAGATGGGCTAG	2447			
	R: GGCTTTGTATATATACACGTG	2448			
55	F: GCAGCATCAAAGACAAGCA	2449	F-E52:	CAAGACCAGCAATCAAGAGGCTAG	2461
	R: TCCTTACGGGTAGCATCC	2450	R-E56:	GAGAGACTTTTTCCGAAGTTCAC	2462

Correction of diverse DMD patient mutations by myoediting

To evaluate the effectiveness of a single-guide RNA to correct different types of human DMD mutations by exon skipping, three DMD iPSC lines with representative types of DMD mutations were obtained: a large deletion (termed Del; lacking exons 48 to 50), a pseudo-exon mutation (termed pEx; caused by an intronic point mutation), and a duplication mutation (termed Dup). Briefly, peripheral blood mono-nuclear cells (PBMCs) obtained from whole blood were cultured and then reprogrammed into iPSCs using recombinant Sendai viral vectors expressing reprogramming factors. Cas9 and guide RNAs for correction or bypass of the mutations in iPSC myoediting on an iPSC line (also known as Del) from a DMD patient with a large deletion of exons 48 to lines were introduced into cells by nucleofection. Pools of treated cells or single clones were then differentiated into induced cardiomyocytes (iCMs) using standardized conditions. Purified iCMs were used to generate 3D-EHM and to perform functional assays (Fig. 2A).

Correction of a large deletion mutation

It is estimated that ~60 to 70% of DMD cases are caused by large deletions of one or more exons. Myoediting was performed on an iPSC line from a DMD patient with a large deletion of exons 48 to 50 in a hotspot. The large deletion creates a frameshift mutation and introduces a premature stop codon in exon 51, as shown in Fig. 2B. Destruction of the splice acceptor in exon 51 will, in principle, allow for splicing of exons 47 to 52, thereby reconstituting the open reading frame (Fig. 2B and Fig. 6B). Theoretically, skipping exon 51 can potentially correct ~13% of DMD patients. Optimized guide RNA Ex51-g3 and Cas9 (Fig. 2C) were nucleofected into this iPSC line, resulting in successful destruction of the splice acceptor or reframing of exon 51 by NHEJ, as demonstrated by genomic sequencing, and restoration of the open reading frame (Fig. 6B). The pool of myoedited and DMD iPSCs (Del-Cor.) was differentiated into iCMs and rescue of in-frame dystrophin mRNA expression was confirmed by sequencing of RT-PCR products from amplification of exons 47 to 52 (Fig. 2D and Fig. 6C).

Correction of a pseudo-exon mutation

To further extend this approach to rare mutations, attempts were made to correct a point mutation within iPSCs from a DMD patient (also known as pEx), who has a spontaneous point mutation in intron 47 (c.6913- 4037T>G). This point mutation generates a novel RNA splicing acceptor site (YnNYAG) and results in a pseudo-exon of exon 47A (Fig. 2E), which encodes

a premature stop signal. Two guide RNAs (Ex47A-g1 and Ex47A-g2) were designed to precisely target the mutation (Fig. 2F and Fig. 7A and 7B). As shown in Fig. 2G, myoediting abolished the cryptic splice acceptor site and permanently skipped the pseudo-exon, restoring full-length dystrophin protein in the corrected cells (pEx-Cor.). The efficacy of exon skipping was tested by RT-PCR in these DMD iCMs (Fig. 2G). Sequencing of the RT-PCR products confirmed that exon 47 was spliced to exon 48 (Fig. 7C).

It is noteworthy that Ex47A-g2 targets only the mutant allele because the wild-type intron lacks the PAM sequence (NAG) for SpCas9. Moreover, the T > G mutation in this patient creates a disease-specific PAM sequence (AG) for Cas9. It is also noteworthy that this type of correction restores the normal dystrophin protein without any internal deletions (Fig. 7B and 7C).

Correction of a large duplication mutation

Exon duplications account for ~10 to 15% of identified DMD-causing mutations. Myoediting was tested on an iPSC line (also known as Dup) from a DMD patient with a large duplication (exons 55 to 59), which disrupts the dystrophin open reading frame (Fig. 2H). Whole-genome sequencing and analysis the copy number variation profile in cells from this patient was performed and identified the precise insertion site in intron 54 (Fig. 2H). This insertion site (In59-In54 junction) was confirmed by PCR (Fig. 8A and Table 4).

It was hypothesized that the 5′ flanking sequence of the duplicated exon 55 is identical such that one guide RNA targeting this region should be able to make two DSBs and delete the entire duplicated region (exons 55 to 59; ~150 kb). To test this hypothesis, three guide RNAs (In54-g1, In54-g2, and In54-g3) were designed to target sequences near the junction of intron 54 and exon 55 (Fig. 2I). The efficiency of DNA cutting with these guide RNAs was evaluated in 293 cells by T7E1 (Fig. 8B). Guide RNA In54-g1 was selected for subsequent experiments on Dup iPSCs. Genomic PCR products from the myoedited Dup iPSC mixture were cloned and sequenced (Fig. 8C).

To confirm the correction of the duplication mutation, the pool of treated DMD iPSCs (also known as Dup-Cor.) was differentiated into cardiomyocytes. mRNA with duplicated exons was semiquantified by RT-PCR using the duplication-specific primers (Ex59F, a forward primer in exon 59, and Ex55R, a reverse primer in exon 55) and normalized to expression of the b-actin gene (Fig. 2J and Table 4). As expected, the duplication-specific RT-PCR band was absent in wild-type (WT) cells and was decreased dramatically in Dup-Cor. cells. To confirm this result, RT-PCR on the duplication borders of exon 53 to Ex55 and Ex59

to exon 60 (Fig. 8D) was performed. The intensity of duplication-specific upper bands was decreased in corrected iCMs. Single colonies were picked from the treated mixture of cells. Duplication-specific PCR primers (F2-R1) were used to screen the corrected colonies (Fig. 8E). PCR results of three representative corrected colonies (Dup-Cor. #4, #6, and #26) and the uncorrected control (Dup) are shown in Fig. 8E. The absence of a duplication-specific PCR band in colonies 4, 6, and 26 confirmed the deletion of the duplicated DNA region.

Restoration of dystrophin protein in patient-derived iCMs by myoediting

Next, the restoration and stable expression of dystrophin protein in single clones and pools of treated iCMs was confirmed by immunocytochemistry (Fig. 3A to 3C, and Figs. 6D, 7D, and 8F) and Western blot analysis (Fig. 24, D to F). Even without clonal selection and expansion, most of the iCMs in Del-Cor., pEx-Cor., and Dup-Cor. were dystrophin-positive (Fig. 3A to 3C, and Figs. 6D, 7D, and 8F). From mixtures of myoedited Del iPSCs, two clones (#16 and #27) were picked and differentiated into cardiomyocytes. Clone #27, which has a higher dystrophin expression level, was selected for subsequent experiments (also known as Del-Cor-SC). One selected clone for corrected pEx (#19) was used for further studies (also known as pEx-Cor-SC). Two selected clones for corrected Dup (#26 and #6) were differentiated into iCMs. Clone #6 was used for functional assay experiments (also known as Dup-Cor-SC). Dystrophin protein expression levels of the corrected iCMs were estimated to be comparable to WT cardiomyocytes (50 to 100%) by immunocytochemistry and Western blot analysis (Fig. 3).

Restoration of function of patient-derived iCMs by myoediting

In addition to measuring dystrophin mRNA and protein expression by biochemical methods, functional analysis to the macroscale was used, using 3D-EHM derived from normal, DMD, and corrected DMD iCMs. Briefly, iPSCs-derived cardiomyocytes were metabolically purified by glucose deprivation. Purified cardiomyocytes were mixed with human foreskin fibroblasts (HFFs) at a 70%:30% ratio. The cell mixture was reconstituted in a mixture of bovine collagen and serum-free medium. After 4 weeks in culture, contraction experiments were performed (Fig. 4A).

EHMs from eight iPSC lines were tested: (i) WT, (ii) uncorrected Del, (iii) Del-Cor-SC, (iv) uncorrected pEx, (v) pEx-Cor., (vi) pEx-Cor-SC, (vii) uncorrected Dup, and (viii) Dup-Cor-SC. Functional phenotyping of DMD and corrected DMD cardiomyocytes in EHM revealed a contractile dysfunction in all DMD EHMs (Del, pEx, and Dup) compared to WT EHMs (Fig. 4B to 4E). A more pronounced contractile dysfunction was seen in Del compared

with pEx and Dup EHM. Force of contraction (FOC) was markedly reduced in DMD EHMs and was significantly improved in corrected DMD EHMs (Del- Cor-SC, pEx-Cor-SC, and Dup-Cor-SC) (Fig. 4B to 4E) with completely restored cardiomyocyte maximal inotropic capacity in Dup-Cor-SC (Fig. 4D and 4E).

Because current gene therapy delivery methods are only able to affect a portion of the heart muscle, an obvious question is what percentage of corrected cardiomyocytes is needed to rescue the phenotype of DCM. To address this question, DMD cells (Del) and corrected DMD cells (Del-Cor-SC) were precisely mixed to simulate a wide range of "therapeutic efficiency" (10 to 100%) in EHM (Fig. 4F). This revealed that 30 to 50% of cardiomyocytes need to be repaired for partial (30%) or maximal (50%) rescue of the contractile phenotype (Fig. 4F). These findings are consistent with previous in vivo studies showing that mosaic dystrophin expression in 50% cardiomyocytes in carrier mice resulted in a near-normal cardiac phenotype. Our findings show that contractile dysfunction was efficiently restored in corrected DMD EHM to a comparable level of WT EHM. Myoediting is thus a highly specific and efficient approach to rescue clinical phenotypes of DMD in EHM.

Discussion

The DMD gene is the largest known gene in the human genome, encompassing 2.6 million base pairs and encoding 79 exons. The large size and complicated structure of the DMD gene contribute to its high rate of spontaneous mutation. There are ~3000 documented mutations in humans, which include large deletions or duplications (~77%), small indels (~12%), and point mutations (~9%). These mutations mainly affect exons; however, intronic mutations can alter the splicing pattern and cause the disease, as shown here for the pEx mutation.

To potentially simplify the correction of diverse DMD mutations by CRISPR/Cas9 gene editing, guide RNAs were identified that are capable of skipping the top 12 exons, which account for ~60% of DMD patients. Thus, it is not necessary to design individual guides for each DMD mutation or excise large genomic regions with pairs of guide RNAs.

Rather, patient mutations can be grouped such that skipping of individual exons can restore dystrophin expression in large numbers of patients. In the proof-of-concept study described in Example 1, the optimized myoediting approach using only one guide RNA efficiently restored the DMD open reading frame in a wide spectrum of mutation types, including large deletions, point mutations, and duplications, which cover most of the DMD

population. Even relatively large and complex deletions can be corrected by a single cut in the DNA sequence that eliminates a splice acceptor or donor site without the requirement for multiple guide RNAs to direct simultaneous cutting at distant sites with ligation of DNA ends. Although exon-skipping mainly converts DMD to milder BMD, for a subset of patients with duplication or pseudo-exon mutations, myoediting can eliminate the mutations and restore the production of normal dystrophin protein, as we have shown in this study for pEx and Dup mutations.

Dilated cardiomyopathy, characterized by contractile dysfunction and ventricular chamber enlargement, is one of the main causes of death in DMD patients. However, because of the marked interspecies differences in cardiac physiology and anatomy, as well as the natural history of the disease, the shortened longevity of these animals (~2 years), and the small size of their hearts (1/3000 the size of the human heart), cardiomyopathy is not generally observed in mouse models of DMD at the young age. To overcome limitations and shortcomings of 2D cell culture systems and small animal models, human iPSC–derived 3D-EHM was used to show that dystrophin mutations impaired cardiac contractility and sensitivity to calcium concentration. Contractile dysfunction was observed in DMD EHM, resembling the DCM clinical phenotype of DMD patients. Contractile dysfunction was partially-to-fully restored in corrected DMD EHM by myoediting. Thus, genome editing represents an effective means of eliminating the genetic cause and correcting the muscle and cardiac abnormalities associated with DMD. The data presented herein further demonstrate that EHM serves as a suitable preclinical tool to approximate therapeutic efficiency of myoediting.

Human CRISPR clinical trials received approval in China and the United States. One key concern for the CRISPR/Cas9 system is specificity because off-target effects may cause unexpected mutations in the genome. Multiple approaches have been developed to evaluate possible off-target effects, including (i) in silico prediction of target sites and testing them by deep sequencing and (ii) unbiased whole-genome sequencing. In addition, several new approaches have been reported to minimize potential off-target effects and/or to improve the specificity of the CRISPR/Cas9 system, including titration of dosage of Cas9 and guide RNA, paired Cas9 nickases, truncated guide RNAs, and high-fidelity or enhanced Cas9. Although most studies have used in vitro cell culture systems, we and others did not observe off-target effects in our previous studies of germline editing and post-natal editing in mice. According to a recent study of gene editing in human preimplantation embryos, off-target mutations were also not detected in the edited genome. Although comprehensive and extensive analysis of off-

target effects is beyond the scope of this study, we are aware that it will eventually be important to thoroughly evaluate possible off-target effects of individual guide RNAs before potential therapeutic application.

Materials and Methods

Plasmids. The pSpCas9(BB)-2A-GFP (PX458) plasmid containing the human codon–optimized SpCas9 gene with 2A-EGFP and the backbone of guide RNA was a gift from F. Zhang (plasmid #48138, Addgene). Cloning of guide RNA was carried out according to the Feng Zhang Lab CRISPR plasmid instructions (addgene.org/crispr/zhang/).

Transfection and cell sorting of human 293 cells. Cells were transfected by Lipofectamine 2000 Transfection Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions, and the cells were incubated for a total of 48 to 72 hours. Cell sorting was performed by the Flow Cytometry Core Facility at University of Texas (UT) Southwestern Medical Center. Transfected cells were dissociated using trypsin-EDTA solution. The mixture was incubated for 5 min at 37°C, and 2 ml of warm Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum was added. The resuspended cells were transferred into a 15-ml Falcon tube and gently triturated 20 times. The cells were centrifuged at 1300 rpm for 5 min at room temperature. The medium was removed, and the cells were resuspended in 500 ml of phosphate-buffered saline (PBS) supplemented with 2% bovine serum albumin (BSA). Cells were filtered into a cell strainer tube through its mesh cap. Sorted single cells were separated into microfuge tubes into GFP+ and GFP- cell populations.

Human iPSC maintenance, nucleofection, and differentiation. The DMD iPSC line Del was purchased from Cell Bank RIKEN BioResource Center (cell no. HPS0164). The WT iPSC line was a gift from D. Garry (University of Minnesota). Other iPSC lines (pEx and Dup) were generated and maintained by UT Southwestern Wellstone Myoediting Core. Briefly, PBMCs obtained from DMD patients' whole blood were cultured and then reprogrammed into iPSCs using recombinant Sendai viral vectors expressing reprogramming factors (Cytotune 2.0, Life Technologies). iPSC colonies were validated by immuno-cytochemistry, mycoplasma testing, and teratoma formation. Human iPSCs were cultured in mTeSRTM1 medium (STEMCELL Technologies) and passaged approximately every 4 days (1:18 split ratio). One hour before nucleofection, iPSCs were treated with 10 mM ROCK inhibitor (Y-27632) and dissociated into single cells using Accutase (Innovative Cell Technologies Inc.). Cells (1×10^6) were mixed with 5 mg of SpCas9-2A-GFP plasmid and nucleofected using the P3 Primary Cell

4D-Nucleofector X kit (Lonza) according to manufacturer's protocol. After nucleofection, iPSCs were cultured in mTeSRTM1 medium supplemented with 10 mM ROCK inhibitor, penicillin-streptomycin (1:100) (Thermo Fisher Scientific), and primosin (100 mg/ml; InvivoGen). Three days after nucleofection, GFP+ and GFP− were sorted by fluorescence-activated cell sorting, as described above, and subjected to PCR and T7E1 assay.

Isolation of genomic DNA from sorted cells. Protease K (20 mg/ml) was added to DirectPCR Lysis Reagent (Viagen Biotech Inc.) to a final concentration of 1 mg/ml. Cells were centrifuged at 4°C at 6000 rpm for 10 min, and the supernatant was discarded. Cell pellets kept on ice were resuspended in 50 to 100 ml of DirectPCR/protease K solution and incubated at 55°C for >2 hours or until no clumps were observed. Crude lysates were incubated at 85°C for 30 min and then spun for 10 s. NaCl was added to a final concentration of 250 mM, followed by the addition of 0.7 volumes of isopropanol to precipitate DNA. The DNA was centrifuged at 4°C at 13,000 rpm for 5 min, and the supernatant was discarded. The DNA pellet was washed with 1 ml of 70% EtOH and dissolved in water. The DNA concentration was measured using a NanoDrop instrument (Thermo Fisher Scientific).

Amplifying targeted genomic regions by PCR. PCR assays contained 2 ml of GoTaq polymerase (Promega), 20 ml of 5× green GoTaq reaction buffer, 8 ml of 25 mM $MgCl_2$, 2 ml of 10 mM primer, 2 ml of 10 mM deoxynucleotide triphosphate, 8 ml of genomic DNA, and double-distilled H_2O (ddH_2O) to 100 ml. PCR conditions were as follows: 94°C for 2 min, 32× (94°C for 15 s, 59°C for 30 s, and 72°C for 1 min), 72°C for 7 min, and then held at 4°C. PCR products were analyzed by 2% agarose gel electrophoresis and purified from the gel using the QIAquick PCR Purification kit (Qiagen) for direct sequencing. These PCR products were subcloned into pCRII-TOPO vector (Invitrogen) according to the manufacturer's instructions. Individual clones were picked, and the DNA was sequenced.

T7E1 analysis of PCR products. Mismatched duplex DNA was obtained by denaturation/renaturation of 25 ml of the genomic PCR samples using the following conditions: 95°C for 10 min, 95° to 85°C (−2.0°C/s), 85°C for 1 min, 85° to 75°C (−0.3°C/s), 75°C for 1 min, 75° to 65°C (−0.3°C/s), 65°C for 1 min, 65° to 55°C (−0.3°C/s), 55°C for 1 min, 55° to 45°C (−0.3°C/s), 45°C for 1 min, 45° to 35°C (−0.3°C/s), 35°C for 1 min, 35° to 25°C (−0.3°C/s), 25°C for 1 min, and then held at 4°C.

Following denaturation/renaturation, the following was added to the samples: 3 ml of 10× NEBuffer 2, 0.3 ml of T7E1 (New England Biolabs), and ddH_2O to 30 ml. Digested reactions were incubated for 1 hour at 37°C. Undigested PCR samples and T7E1-digested PCR

products were analyzed by 2% agarose gel electrophoresis.

Whole-genome sequencing. Whole-genome sequencing was performed by submitting the blood samples to Novogene Corporation. Purified genomic DNA (1.0 mg) was used as input material for the DNA sample preparation. Sequencing libraries were generated using TruSeq Nano DNA HT Sample Preparation kit (Illumina) following the manufacturer's instructions. Briefly, the DNA sample was fragmented by sonication to a size of 350 bp. The DNA fragments were end-polished, A-tailed, and ligated with the full-length adapter for Illumina sequencing with further PCR amplification. The libraries were sequenced on an Illumina sequencing platform, and paired-end reads were generated.

Isolation of RNA. RNA was isolated from cells using TRIzol RNA isolation reagent (Thermo Fisher Scientific) according to the manufacturer's instructions.

Cardiomyocyte differentiation and purification. iPSCs were adapted and maintained in TESR-E8 (STEMCELL Technologies) on 1:120 Matrigel in PBS-coated plates and passaged using EDTA solution (Versene, Thermo Fisher Scientific) twice weekly. For cardiac differentiation, iPSCs were plated at 5×10^4 to 1×10^5 cells/cm^2 and induced with RPMI, 2% B27, 200 mM L-ascorbic acid-2-phosphate sesquimagnesium salt hydrate (Asc; Sigma-Aldrich), activin A (9 ng/ml; R&D Systems), BMP4 (5 ng/ml; R&D Systems), 1 mM CHIR99021 (Stemgent), and FGF-2 (5 ng/ml; Miltenyi Biotec) for 3 days; following another wash with RPMI medium, cells were cultured from days 4 to 13 with 5 mM IWP4 (Stemgent) in RPMI supplemented with 2% B27 and 200 mM Asc. Cardiomyocytes were metabolically purified by glucose deprivation from days 13 to 17 in glucose-free RPMI (Thermo Fisher Scientific) with 2.2 mM sodium lactate (Sigma-Aldrich), 100 mM b-mercaptoethanol (Sigma-Aldrich), penicillin (100 U/ml), and streptomycin (100 mg/ml). Cardiomyocyte purity was 92 ± 2% from 15 independent differentiation runs (one to three for each cell line).

EHM generation. To generate defined, serum-free EHM, purified cardiomyocytes were mixed with HFFs (American Type Culture Collection) at a 70%:30% ratio. The cell mixture was reconstituted in a mixture of pH-neutralized medical-grade bovine collagen (0.4 mg per EHM; LLC Collagen Solutions) and concentrated serum-free medium [2× RPMI, 8% B27 without insulin, penicillin (200 U/ml), and streptomycin (200 mg/ml)] and cultured for 3 days in Iscove medium with 4% B27 without insulin, 1% nonessential amino acids, 2 mM glutamine, 300 mM ascorbic acid, IGF1 (100 ng/ml; AF-100-11), FGF-2 (10 ng/ml; AF-100-18B), VEGF165 (5 ng/ml; AF-100-20), TGF-b1 (5 ng/ml; AF-100-21C; all growth factors are from PeproTech), penicillin (100 U/ml), and streptomycin (100 mg/ml). After a 3-day

condensation period, EHM were transferred to flexible holders to support auxotonic contractions. Analysis was carried out after a total EHM culture period of 4 weeks.

Analysis of contractile function. Contraction experiments were performed under isometric conditions in organ baths at 37°C in gassed (5% CO_2/95% O_2) Tyrode's solution (containing 120 mM NaCl, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 5.4 mM KCl, 22.6 mM $NaHCO_3$, 4.2 mM NaH_2PO_4, 5.6 mM glucose, and 0.56 mM ascorbate). EHM were electrically stimulated at 1.5 Hz with 5-ms square pulses of 200 mA. EHMs were mechanically stretched at intervals of 125 mm until the maximum systolic force amplitude (FOC) was observed according to the Frank-Starling law. Responses to increasing extracellular calcium (0.2 to 4 mM) were investigated to determine maximal inotropic capacity. Where indicated, forces were normalized to muscle content (sarcomeric a-actinin–positive cell content, as determined by flow cytometry).

Flow cytometry of EHM-derived cells. Single-cell suspensions of EHM were prepared as described previously and fixed in 70% ice-cold ethanol. Fixed cells were stained with Hoechst 3342 (10 mg/ml; Life Technologies) to exclude cell doublets. Cardiomyocytes were identified by sarcomeric a-actinin staining (clone EA-53, Sigma-Aldrich). Cells were run on a LSRII SORP cytometer (BD Biosciences) and analyzed using the DIVA software. At least 10,000 events were analyzed per sample.

Immunostaining. iPSC-derived cardiomyocytes were fixed with acetone and subjected to immunostaining. Fixed cardiomyocytes were blocked with serum cocktail (2% normal horse serum/2% normal donkey serum/0.2% BSA/PBS), and incubated with dystrophin antibody (1:800; MANDYS8, Sigma-Aldrich) and troponin-I antibody (1:200; H170, Santa Cruz Biotechnology) in 0.2% BSA/PBS. Following overnight incubation at 4°C, they were incubated with secondary antibodies [biotinylated horse anti-mouse immunoglobulin G (IgG) (1:200; Vector Laboratories) and fluorescein-conjugated donkey anti-rabbit IgG (1:50; Jackson ImmunoResearch)] for 1 hour. Nuclei were counter-stained with Hoechst 33342 (Molecular Probes).

EHM cryosections to be immunostained were thawed, further air-dried, and fixed in cold acetone (10 min at −20°C). Sections were briefly equilibrated in PBS (pH 7.3) and then blocked for 1 hour with serum cocktail (2% normal horse serum/2% normal donkey serum/0.2% BSA/ PBS). Blocking cocktail was decanted, and dystrophin/troponin primary antibody cocktail [mouse anti-dystrophin, MANDYS8 (1:800; Sigma- Aldrich) and rabbit anti–troponin-I (1:200; H170, Santa Cruz Bio- technology)] in 0.2% BSA/PBS was applied without

intervening wash. Following overnight incubation at 4°C, unbound primary antibodies were removed with PBS washes, and sections were probed for 1 hour with secondary antibodies [biotinylated horse anti-mouse IgG (1:200; Vector Laboratories) and rhodamine donkey anti-rabbit IgG (1:50; Jackson ImmunoResearch)] diluted in 0.2% BSA/PBS. Unbound secondary antibodies were removed with PBS washes, and final dystrophin labeling was carried out with a 10-min incubation of the sections with fluorescein-avidin-DCS (1:60; Vector Laboratories) diluted in PBS. Unbound rhodamine was removed with PBS washes, nuclei were counterstained with Hoechst 33342 (2 mg/ml; Molecular Probes), and slides were coverslipped with Vectashield (Vector Laboratories).

Western blot analysis. Western blot analysis for human iPSC–derived cardiomyocytes was performed, using antibodies to dystrophin (ab15277, Abcam; D8168, Sigma-Aldrich), glyceraldehyde-3-phosphate dehydrogenase (MAB374, Millipore), and cardiac myosin heavy chain (ab50967, Abcam). Goat anti-mouse and goat anti-rabbit horseradish peroxidase–conjugated secondary antibodies (Bio-Rad) were used for described experiments.

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All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the disclosure. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure as defined by the appended claims.

VIII. References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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WHAT IS CLAIMED:

1. A method for editing a mutant dystrophin gene in a cardiomyocyte, the method comprising contacting the cardiomyocyte with:

a Cas9 nuclease, or a sequence encoding a Cas9 nuclease, and

a gRNA, or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene.

2. The method of claim 1, wherein the gRNA targets a splice donor or splice acceptor site of exon 51, 45, 53, 44, 46, 52, 50, 43, 6, 7, 8, or 55.

3. The method of claim 1 or claim 2, wherein the gRNA comprises or targets a sequence of any one of SEQ ID NOs. 60-705, 712-862, 947-2377.

4. The method of any one of claims 1-3, wherein a vector comprises the gRNA, or a sequence encoding the gRNA.

5. The method of claim 4, wherein the vector is a viral vector or a non-viral vector.

6. The method of claim 5, wherein the viral vector is an adeno-associated viral (AAV) vector.

7. The method of claim 6, wherein the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV.

8. The method of claim 5, wherein the non-viral vector is a plasmid.

9. The method of claim 5, wherein the non-viral vector is a nanoparticle.

10. The method of any one of claims 1-9, wherein a first vector comprises the gRNA, or a sequence comprising the gRNA, and a second vector comprises the Cas9, or a sequence comprising the Cas9.

11. The method of claim 10, wherein the first vector and the second vector are AAVs.

12. The method of any one of claims 1-11, wherein the mutant dystrophin gene comprises a point mutation.

13. The method of claim 12, wherein the point mutation is a pseudo-exon mutation.

14. The method of claim any one of claims 1-13, wherein the mutant dystrophin gene comprises a deletion.

15. The method of any one of claims 1-14, wherein the mutant dystrophin gene comprises a duplication mutation.

16. The method of any one of claims 1-15, wherein the Cas9 nuclease is isolated or derived from a Streptococcus pyogenes (spCas9).

17. The method of any one of claims 1-15, wherein the Cas9 nuclease is isolated or derived from a Staphylococcus aureus (saCas9).

18. A cardiomyocyte produced according to the method of any one of claims 1-17, wherein the cardiomyocyte expresses a dystrophin protein.

19. The cardiomyocyte of claim 18, wherein the cardiomyocyte is derived from an induced pluripotent stem cell (iPSC).

20. A composition comprising a therapeutically effective amount of the cardiomyocyte of claim 18 or claim 19.

21. A method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a composition of claim 20.

22. The method of claim 21, wherein the therapeutically effective amount at least partially or completely restores cardiac contractility in the patient.

23. An induced pluripotent stem cell (iPSC) comprising:
a Cas9 nuclease, or a sequence encoding a Cas9 nuclease, and
a gRNA, or a sequence encoding a gRNA,
wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene.

24. A composition comprising a cardiomyocyte derived from the iPSC of claim 23.

25. A method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the composition of claim 24.

26. The method of claim 25, wherein the therapeutically effective amount at least partially or completely restores cardiac contractility in the patient.

27. A method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising administering to the subject:
a Cas9 nuclease, or a sequence encoding a Cas9 nuclease, and
a gRNA, or a sequence encoding a gRNA, wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene;
wherein the administering restores dystrophin expression in at least 10% of the subject's cardiomyocytes.

28. The method of claim 27, wherein the gRNA targets a splice donor or splice acceptor site of exon 51, 45, 53, 44, 46, 52, 50, 43, 6, 7, 8, or 55.

29. The method of claim 27 or claim 28, wherein the gRNA comprises or targets a sequence of any one of SEQ ID NOs. 60-705, 712-862, or 947-2377.

30. The method of any one of claims 27-29, wherein a vector comprises the gRNA, or a sequence encoding the gRNA.

31. The method of claim 30, wherein the vector is a viral vector or a non-viral vector.

32. The method of claim 31, wherein the viral vector is an adeno-associated viral (AAV) vector.

33. The method of claim 32, wherein the AAV vector is selected from an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVRh74, AAV2i8, AAVRh10, AAV39, AAV43, AAVRh8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV.

34. The method of claim 31, wherein the non-viral vector is a plasmid.

35. The method of claim 31, wherein the non-viral vector is a nanoparticle.

36. The method of any one of claims 27-35, wherein a first vector comprises the gRNA, or a sequence encoding the gRNA, and a second vector comprises the Cas9, or a sequence encoding the Cas9.

37. The method of claim 36, wherein the first vector and the second vector are AAVs.

38. The method of any one of claims 27-37, wherein the mutant dystrophin gene comprises a point mutation.

39. The method of claim 38, wherein the point mutation is a pseudo-exon mutation.

40. The method of any one of claims 27-39, wherein the mutant dystrophin gene comprises a deletion.

41. The method of any one of claims 27-40, wherein the mutant dystrophin gene comprises a duplication mutation.

42. The method of any one of claims 27-41, wherein the Cas9 nuclease is isolated or derived from a Streptococcus pyogenes (spCas9).

43. The method of any one of claims 27-42, wherein the Cas9 nuclease is isolated or derived from a Staphlyococcus aureus Cas9 (saCas9).

44. The method of any one of claims 27-43, wherein the subject suffers from dilated cardiomyopathy.

45. The method of any one of claims 27-44, wherein the administering restores dystrophin expression in at least 30% of the subject's cardiomyocytes.

46. The method of any one of claims 27-45, wherein the administering at least partially rescues cardiac contractility.

47. The method of any one of claims 27-46, wherein the administering restores dystrophin expression in at least 50% of the subject's cardiomyocytes.

48. The method of any one of claims 27-47, wherein the administering completely rescues cardiac contractility.

49. A method for treating or preventing Duchene Muscular Dystrophy (DMD) in a subject in need thereof, the method comprising:

contacting an induced pluripotent stem cell (iPSC) with

a Cas9 nuclease, or a sequence encoding a Cas9 nuclease, and

a gRNA, or a sequence encoding a gRNA,

wherein the gRNA targets a splice donor or splice acceptor site of the dystrophin gene;

differentiating the iPSC into a cardiomyocyte; and

administering the cardiomyocyte to the subject.

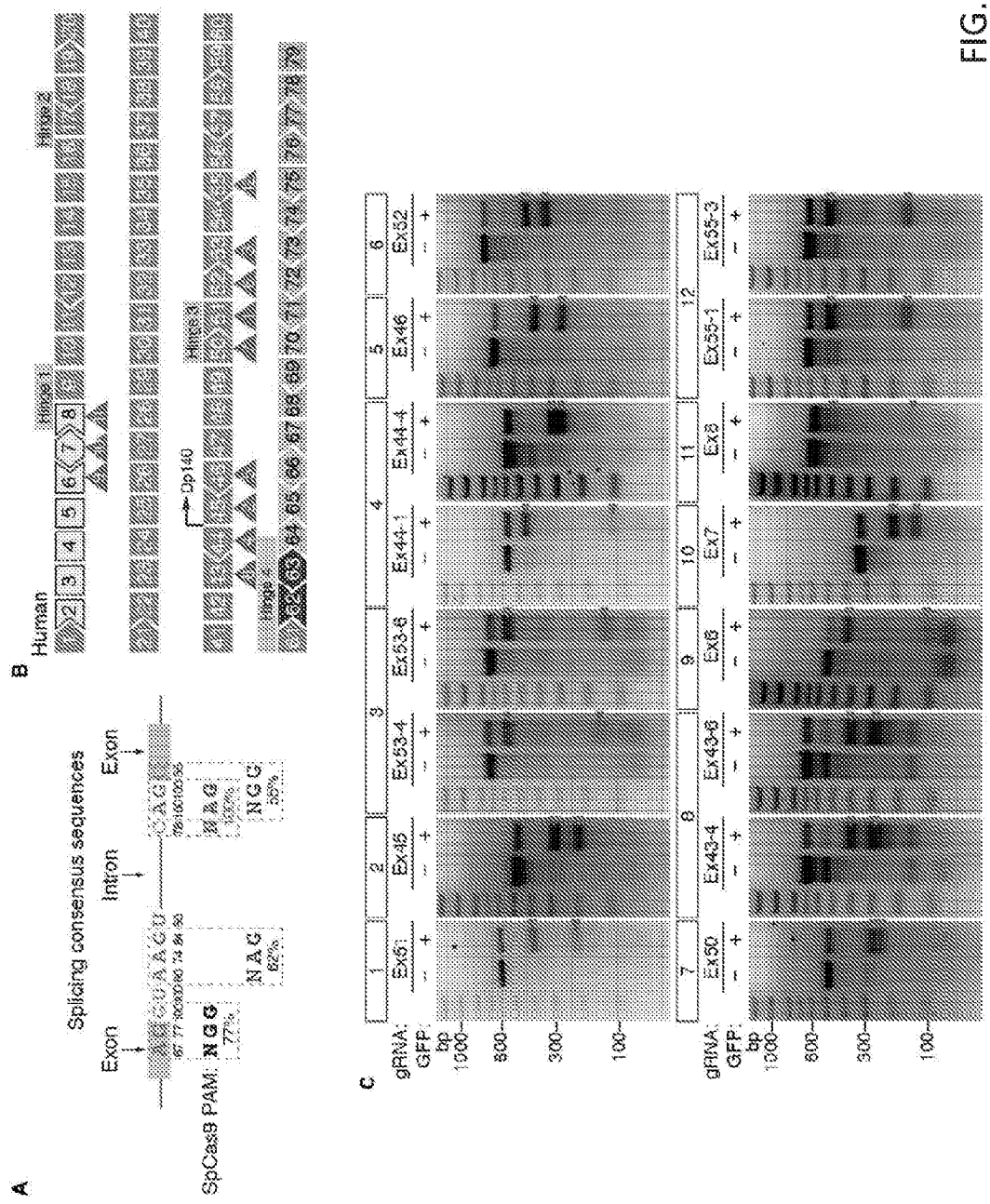
A
Splicing consensus sequences
Exon
Intron
Exon
SpCas9 PAM:
NGG
77%
NAG
82%
NAG
82%
NGG
82%
B
Human
Hinge 1
Hinge 2
Hinge 3
Hinge 4
Dp140
C
gRNA:
GFP:
Ex51
Ex45
Ex53-4
Ex53-6
Ex44-1
Ex44-4
Ex46
Ex52
Ex50
Ex43-4
Ex43-6
Ex8
Ex7
Ex8
Ex55-1
Ex55-3
bp
1000
800
300
100

FIG. 1A-1C

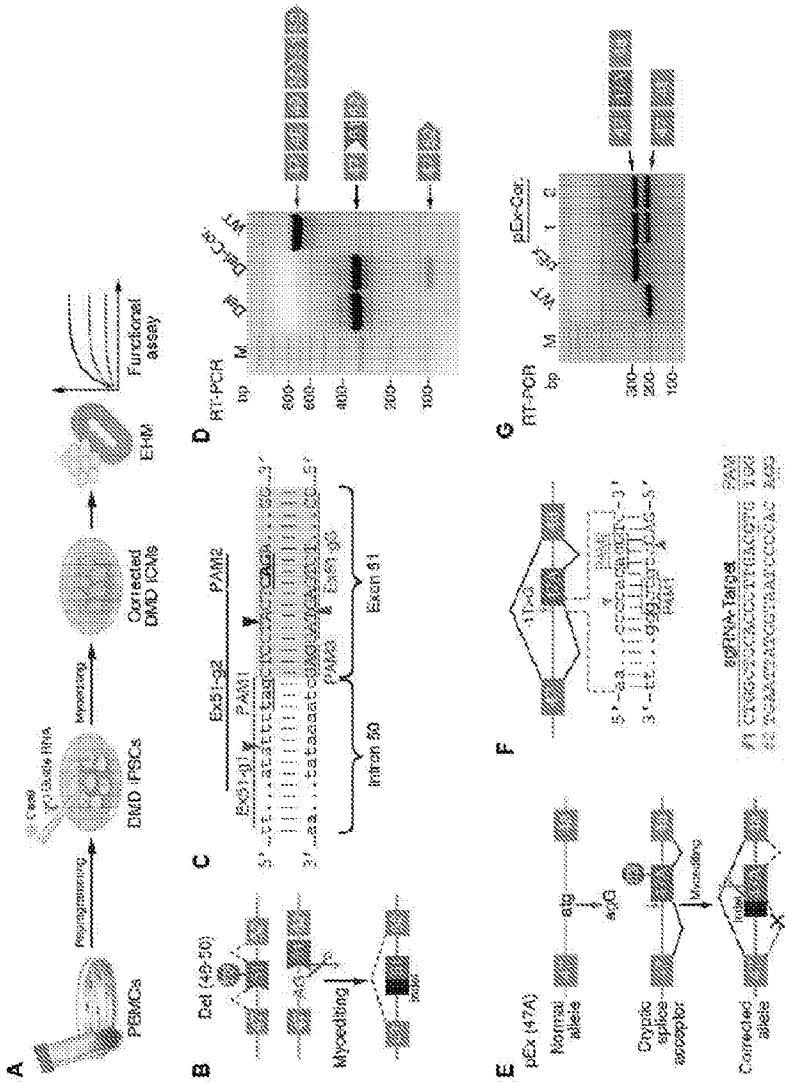
A
PBMCs
Reprogramming
iPS cells
DMD iPSCs
Correcting
Corrected DMD iCMs
EHM
Functional assay
B
Del (49-50)
Myoediting
C
Ex51-g2
PAM2
Ex51-g1
PAM1
PAM2
Exon 50
Exon 51
Intron 50
D
RT-PCR
bp
600-
400-
200-
100-
E
pEx (47A)
Normal allele
atg
stop
Cryptic splice acceptor
Myoediting
Corrected allele
F
sgRNA Target
PAM
G
RT-PCR
bp
300-
200-
100-

FIG. 2A-2G

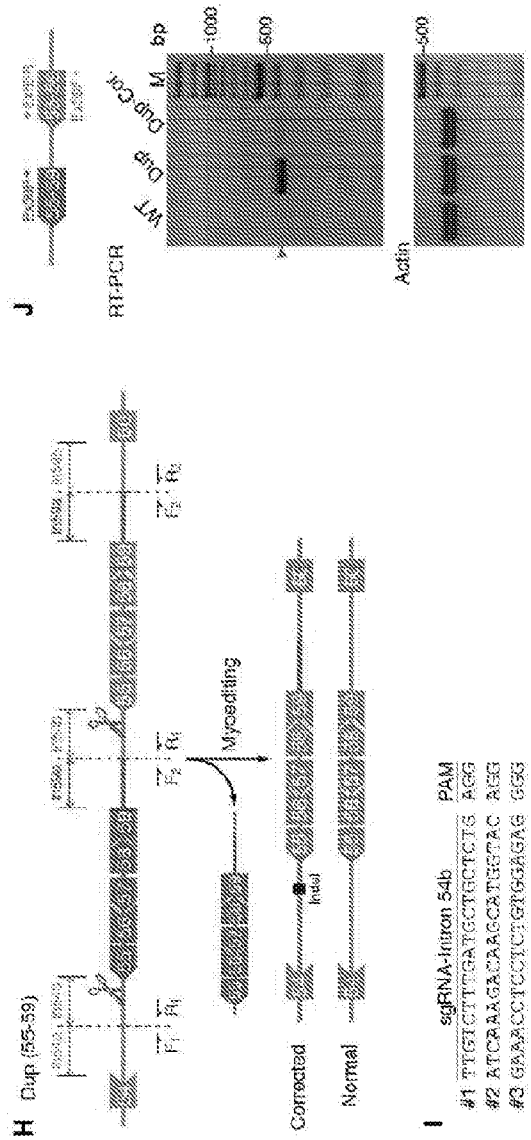
J
RT-PCR
WT
Dup
Dup Corr.
M
bp
1000
600
Actin
500
H
Dup (55-59)
Myoediting
Indel
Corrected
Normal
I
sgRNA-Intron 54b
PAM
#1 TTGTCTTTGATGCTGCTCTG AGG
#2 ATCAAAGACAAGCATGCTAC AGG
#3 GAAACCTCCTCTGTGGAGAG GGG

FIG. 2H-2J

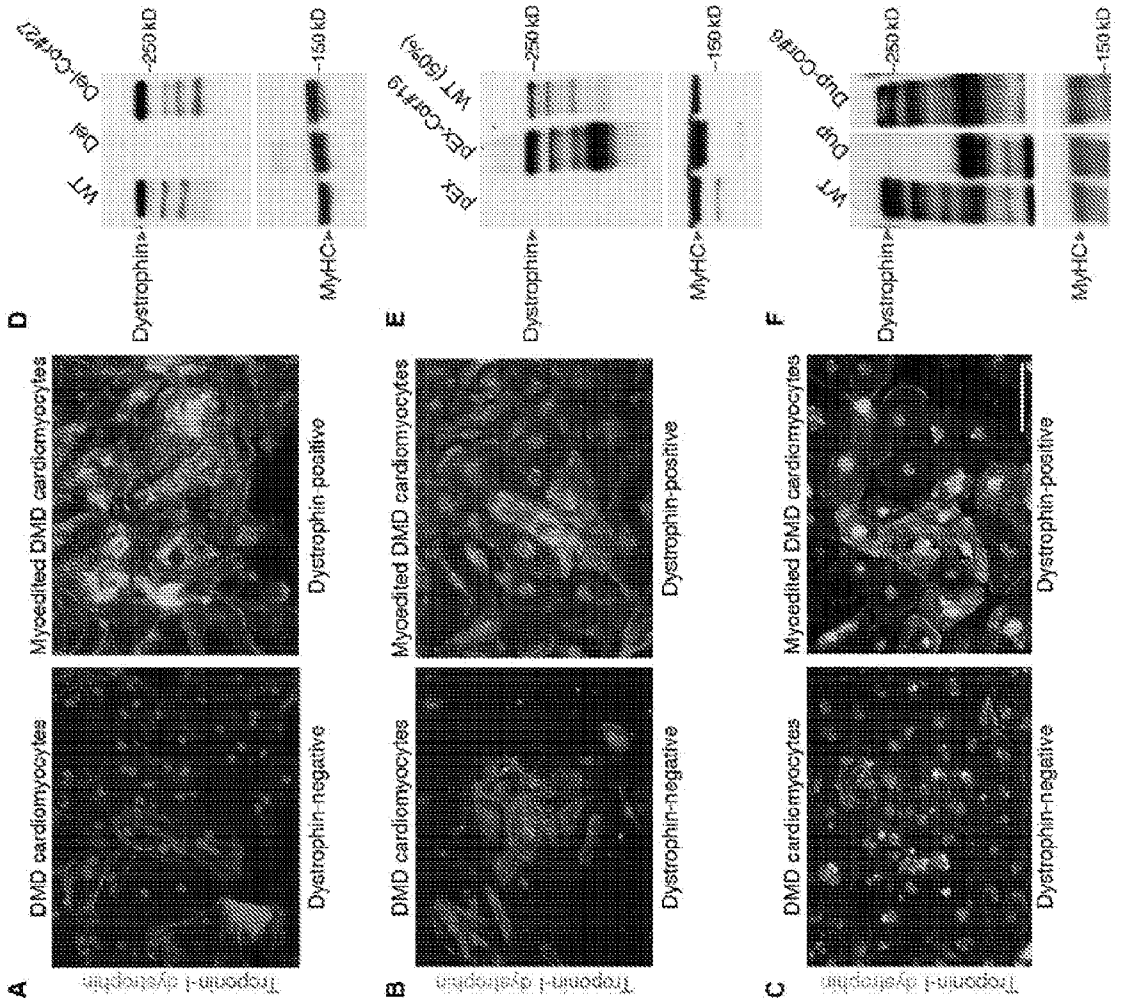
A
Troponin-I dystrophin
DMD cardiomyocytes
Dystrophin-negative
Myoedited DMD cardiomyocytes
Dystrophin-positive
B
Troponin-I dystrophin
DMD cardiomyocytes
Dystrophin-negative
Myoedited DMD cardiomyocytes
Dystrophin-positive
C
Troponin-I dystrophin
DMD cardiomyocytes
Dystrophin-negative
Myoedited DMD cardiomyocytes
Dystrophin-positive
D
WT
Dup
Dup-Corr#21
~250 kD
Dystrophin
~150 kD
MyHC
E
WT (50%)
DEX-Corr#19
DEX
~250 kD
Dystrophin
~150 kD
MyHC
F
WT
Dup
Dup-Corr#6
~250 kD
Dystrophin
~150 kD
MyHC

FIG. 3A-3F

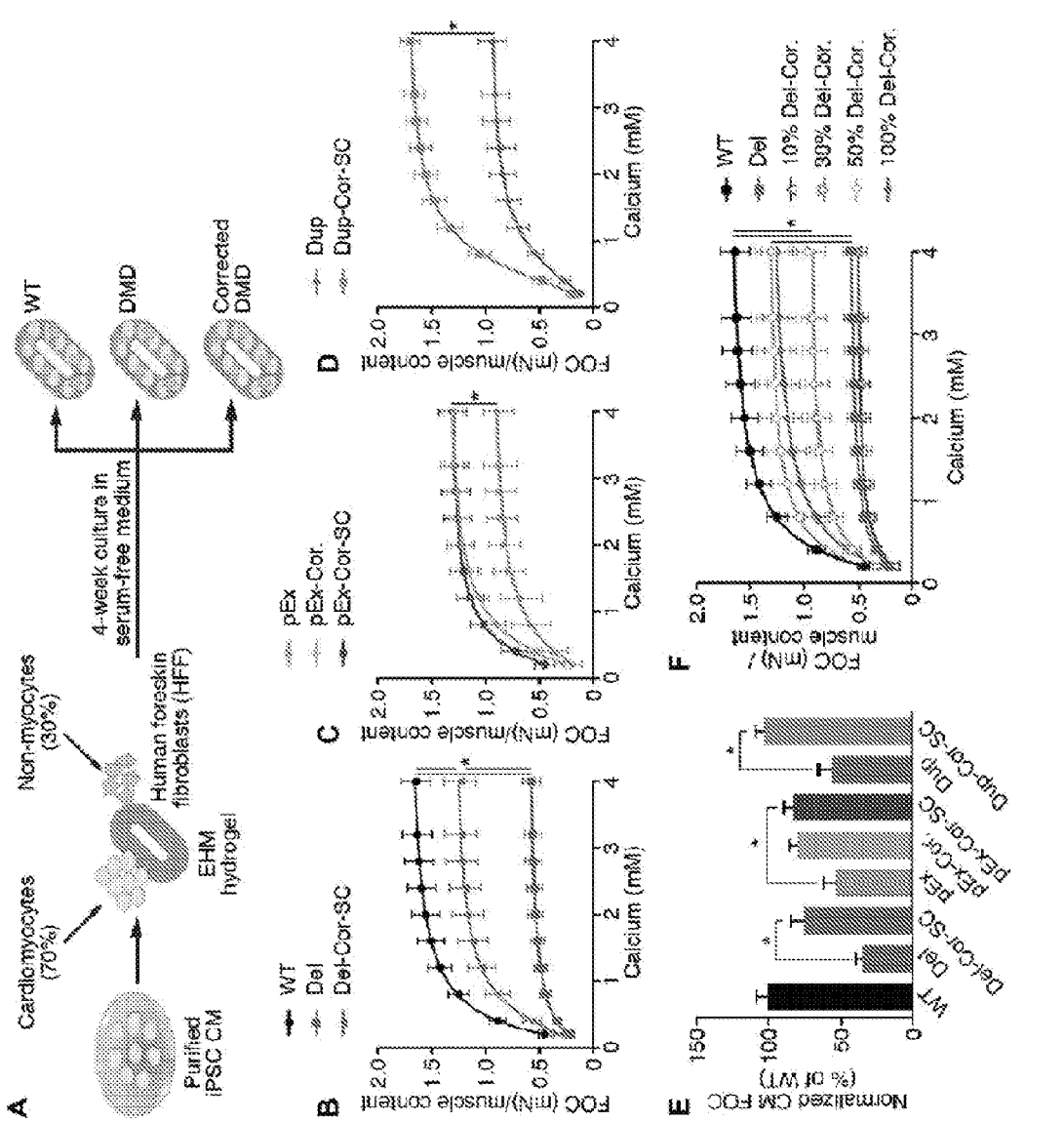
A
Cardiomyocytes (70%)
Non-myocytes (30%)
Human foreskin fibroblasts (HFF)
EHM hydrogel
Purified iPSC CM
4-week culture in serum-free medium
WT
DMD
Corrected DMD
B
WT
Del
Del-Cor-SC
FOC (mN)/muscle content
Calcium (mM)
C
pEx
pEx-Cor.
pEx-Cor-SC
D
Dup
Dup-Cor-SC
E
Normalized CM FOC (% of WT)
WT
Del
Del-Cor-SC
pEx
pEx-Cor.
pEx-Cor-SC
Dup
Dup-Cor-SC
F
FOC (mN) / muscle content
WT
Del
10% Del-Cor.
30% Del-Cor.
50% Del-Cor.
100% Del-Cor.

FIG. 4A-4F

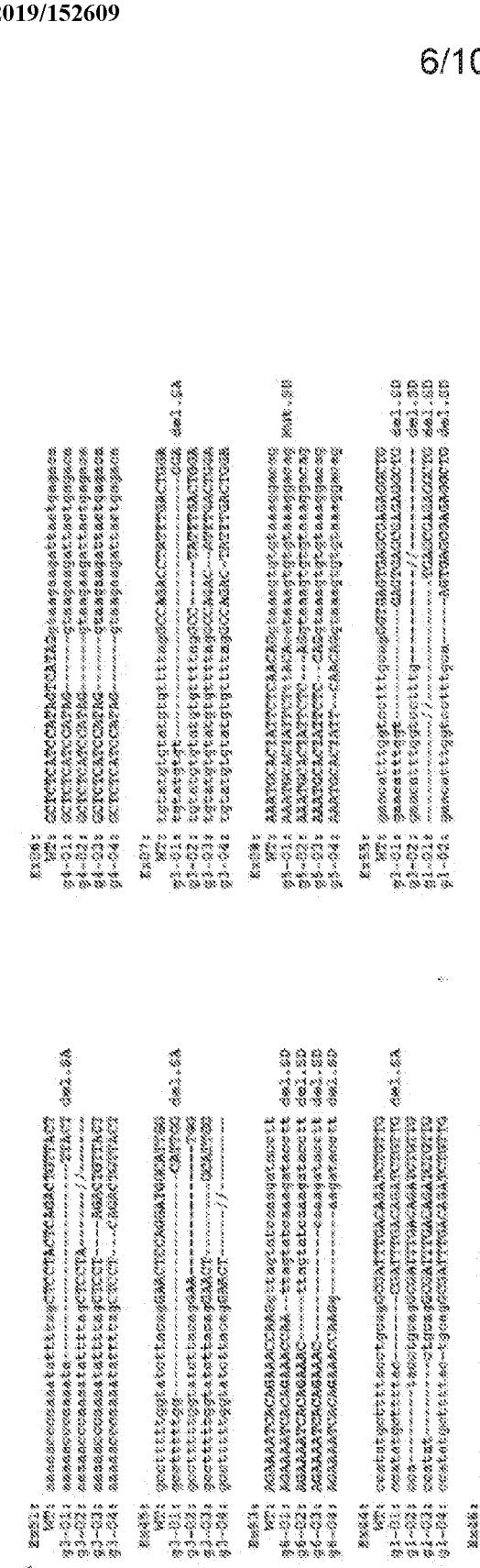

FIG. 5A

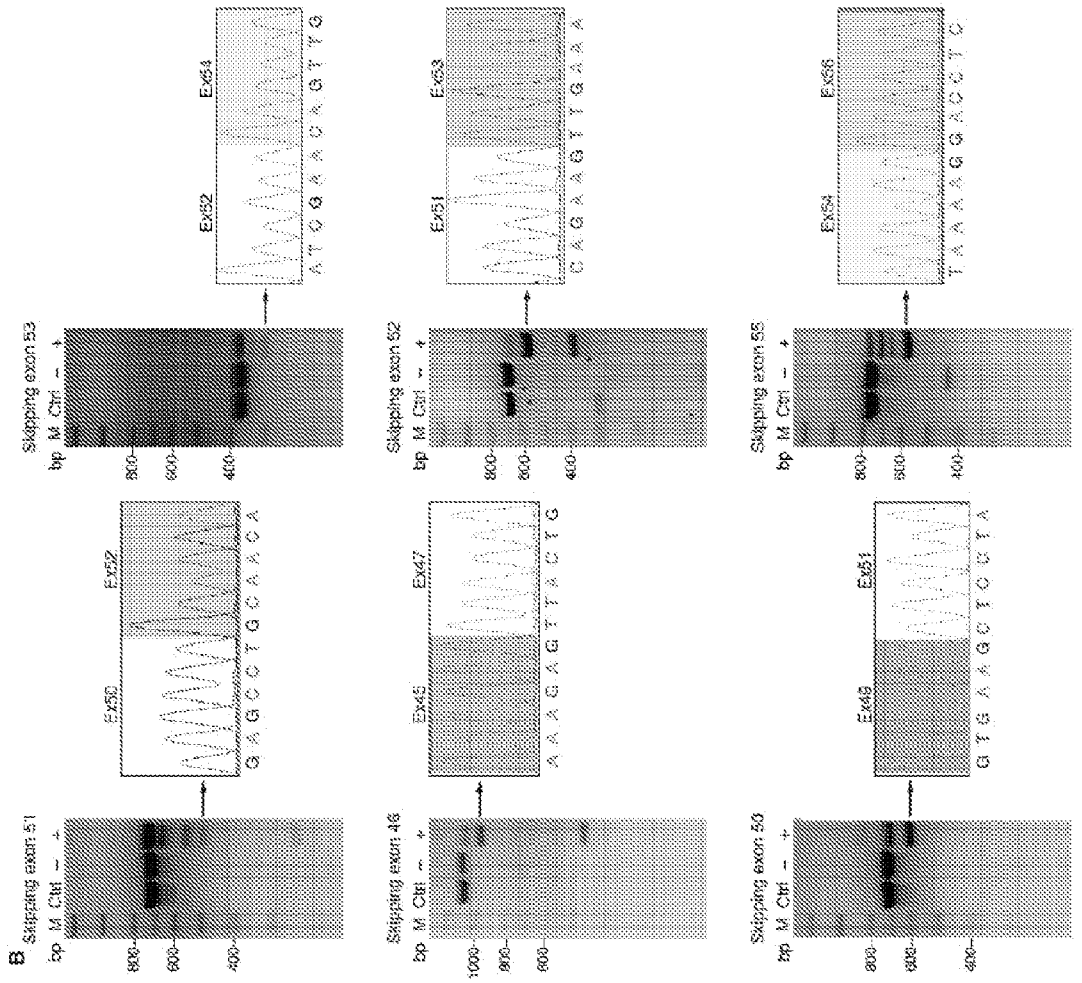
B
Skipping exon 51
bp M Ctrl − +
800
600
400
Ex50
Ex52
G A G C C T G C A A C A
Skipping exon 53
bp M Ctrl − +
800
600
400
Ex52
Ex54
A T C G A A C A G T T G
Skipping exon 46
bp M Ctrl − +
1000
800
600
Ex45
Ex47
A A A G A G T T A C T G
Skipping exon 52
bp M Ctrl − +
800
600
400
Ex51
Ex53
C A G A A G T T G A A A
Skipping exon 50
bp M Ctrl − +
800
600
400
Ex49
Ex51
G T T G A A G C T C C T A
Skipping exon 55
bp M Ctrl − +
800
600
400
Ex54
Ex56
T A A A A G G A C G T C

FIG. 5B

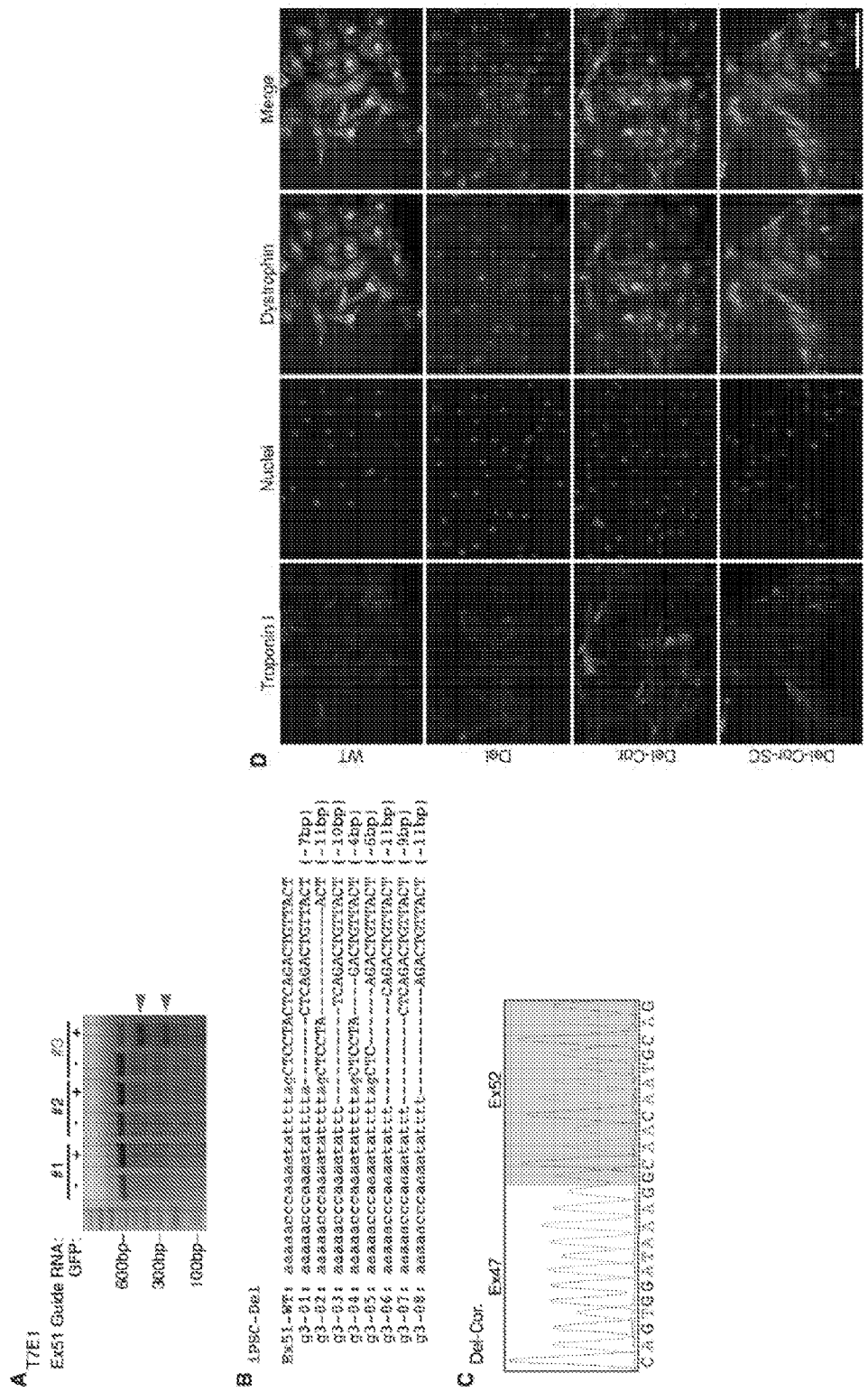
A
T7E1
Ex51 Guide RNA:
#1
#2
#3
GFP:
600bp
300bp
100bp
B
19SC-Bel
Ex51-WT:
g3-01:
g3-02:
g3-03:
g3-04:
g3-05:
g3-06:
g3-07:
g3-08:
(-7bp)
(-11bp)
(-10bp)
(-4bp)
(-6bp)
(-11bp)
(-4bp)
(-11bp)
C
Del-Cor.
Ex47
Ex52
D
WT
Del
Del-Cor
Del-Cor-SC
Troponin I
Nuclei
Dystrophin
Merge

FIG. 6A-6D

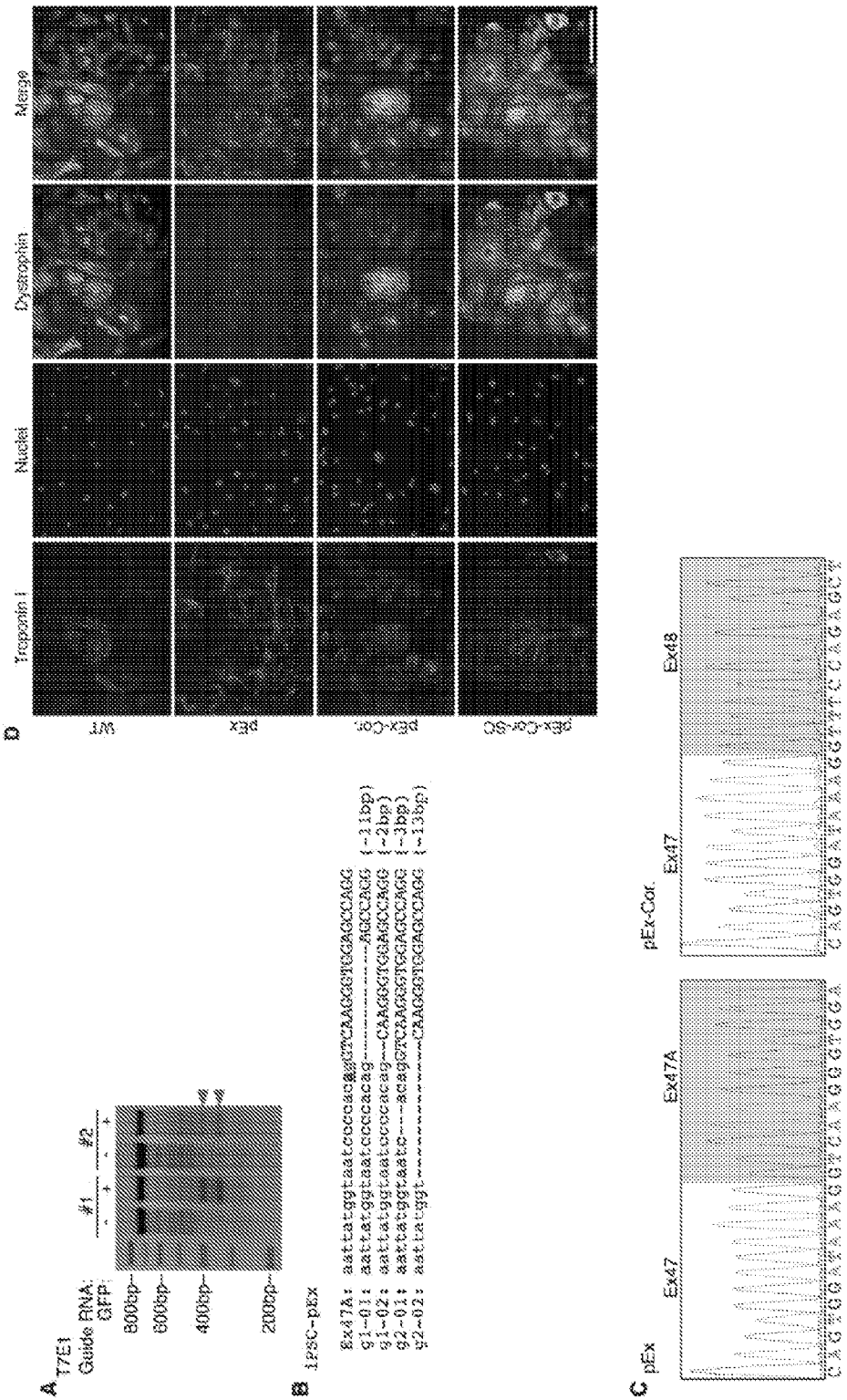
A
T7E1
Guide RNA:
GFP
#1
#2
800bp
600bp
400bp
200bp
B
iPSC-pEx
Ex47A: aattatggtaatcccacagGTCAAGGGTGGAGCCAGG
g1-01: aattatggtaatccccacag----------AGCCAGG (-11bp)
g1-02: aattatggtaatccccacag---CAAGGGTGGAGCCAGG (-2bp)
g2-01: aattatggtaatc---acagGTCAAGGGTGGAGCCAGG (-3bp)
g2-02: aattatggt----------CAAGGGTGGAGCCAGG (-13bp)
C
pEx
Ex47
Ex47A
CAGTGGAATAAAGGTCAAGGGTGGGA
pEx-Cor.
Ex47
Ex48
CAGTGGAATAAAGGTTTCCAGAGCT
D
Troponin I
Nuclei
Dystrophin
Merge
WT
pEx
pEx-Cor.
pEx-Cor.-SC

FIG. 7A-7D

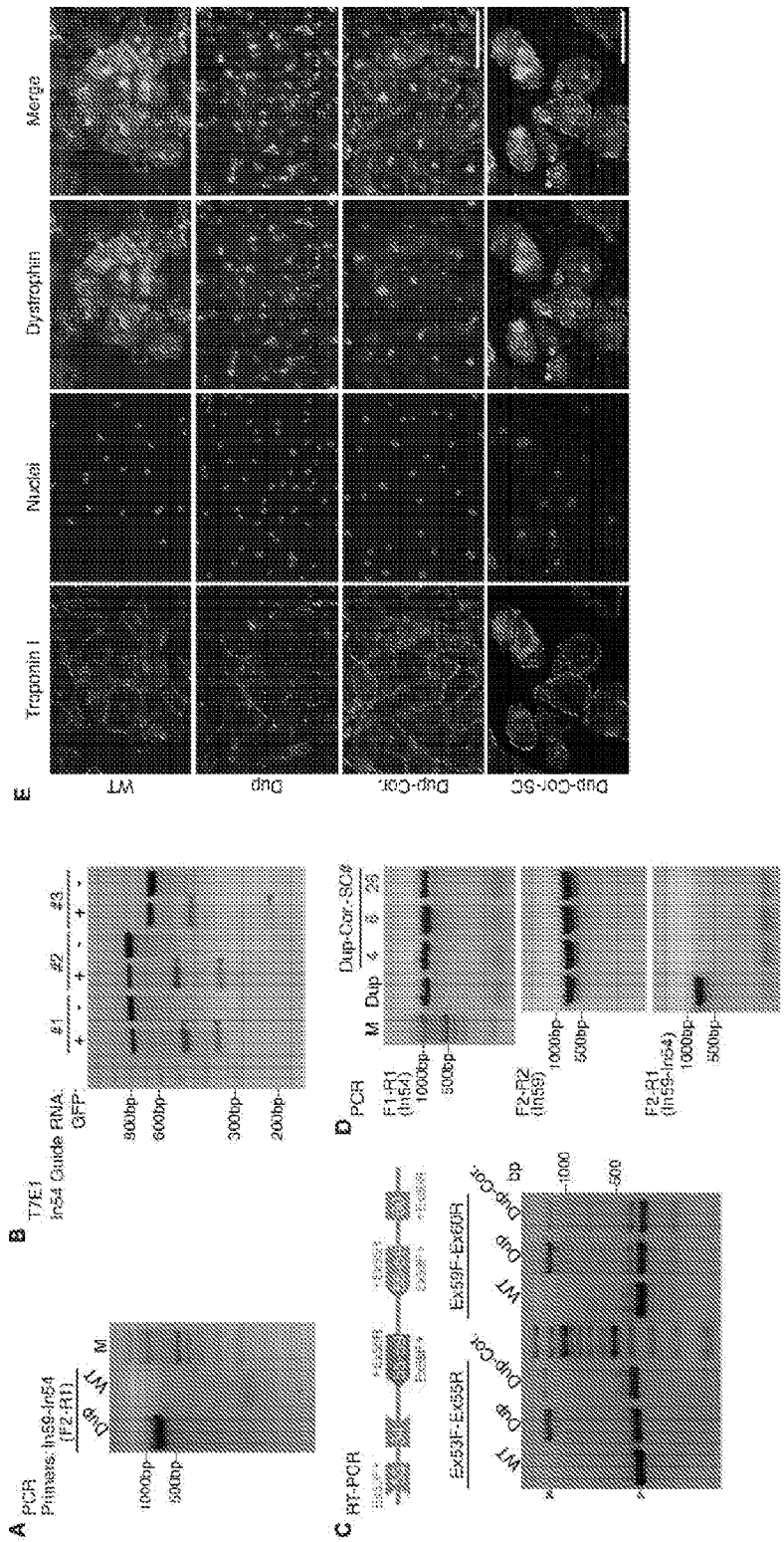
A
PCR
Primers: In59-In54 (F2-R1)
WT
Dup
M
1000bp
500bp
B
T7E1
In54 Guide RNA:
GFP
#1
#2
#3
900bp
600bp
300bp
200bp
C
RT-PCR
Ex53F-Ex55R
Ex59F-Ex60R
WT
Dup
Dup-Cor.
bp
1000
500
D
PCR
M
Dup
Dup-Cor.-SC#
4
8
25
F1-R1 (In54)
1000bp
500bp
F2-R2 (In59)
1000bp
500bp
F2-R1 (In59-In54)
1000bp
500bp
E
Troponin I
Nuclei
Dystrophin
Merge
WT
Dup
Dup-Cor.
Dup-Cor.-SC

FIG. 8A-8E