The lncRNA H19 alleviates muscular dystrophy by stabilizing dystrophin

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Dystrophin proteome regulation in muscular dystrophies (MDs) remains unclear. We report that a long noncoding RNA (lncRNA), H19, associates with dystrophin and inhibits E3-ligase-dependent polyubiquitination at Lys 3584 (referred to as Ub-DMD) and its subsequent protein degradation. In-frame deletions in BMD and a DMD non-silent mutation (C3340Y) result in defects in the ability of the protein to interact with H19, which caused elevated Ub-DMD levels and dystrophin degradation. Dmd C3333Y mice exhibited progressive MD, elevated serum creatine kinase, heart dilation, blood vessel irregularity and respiratory failure with concurrently reduced dystrophin and increased Ub-DMD status. H19 RNA oligonucleotides conjugated with agrin (AGR–H19) and nifenazone competed with or inhibited TRIM63. Dmd C3333Y animals, induced-pluripotent-stem-cell-derived skeletal muscle cells from patients with Becker MD and mdx mice subjected to exon skipping exhibited inhibited dystrophin degradation, preserved skeletal and cardiac muscle histology, and improved strength and heart function following AGR–H19 or nifenazone treatment. Our study paves the way for meaningful targeted therapeutics for Becker MD and for certain patients with Duchenne MD.

Muscular dystrophies (MDs) are a heterogeneous group of inherited diseases of skeletal, smooth and cardiac muscle that cause progressive weakness and degeneration of muscle fibres. Although a number of factors have been linked to different types of MDs, disrupting (DMD) or non-disrupting (BMD) mutations in the reading frame of the gene encoding dystrophin (DMD) are the causative genetic defects of these diseases1, which result in the absence or reduced level of the 427-kDa protein. While Duchenne MD (DMD) and Becker MD (BMD) are considered X-linked recessive disorders14, approximately 8% of female patients with DMD exhibit muscle weakness15. A phenotype-based frame-type analysis held true for 92% of in-frame deletions in 1,024 patients with Becker MD and for 10% of in-frame deletions in 2,688 patients with DMD16. A cohort of point mutations in the DMD gene also leads to a decrease in dystrophin protein levels in vivo17. The absence or reduction of dystrophin could be the consequence of genomic, epigenomic, transcriptomic and proteomic pathways.

The importance of long noncoding RNAs (lncRNAs) in regulating homeostasis and inherited diseases is largely unknown. Here, we report that the lncRNA H19 associates with dystrophin at carboxy termini. H19–dystrophin interactions inhibit TRIM63-dependent K48-linked polyubiquitination (polyUb) of dystrophin at Lys 3584 (Ub-DMD), which prevents protein degradation. Human induced pluripotent stem cells (hiPSCs) derived from patients with BMD exhibit elevated Ub-DMD levels in iPSC-differentiated skeletal muscle cells (SkMCs) and cardiomyocytes (CMs), which is reversed following expression of H19. Mice harbouring the Dmd C3333Y mutant, which models DMD C3340Y, exhibit progressive MD, elevated serum creatine kinase (CK), heart dilation, blood vessel irregularity and respiratory failure. We determine the minimal lncRNA sequence of H19 required to compete with TRIM63–dystrophin interactions and conjugate it with a muscle-enriching peptide, which resulted in the development of agrin–H19 (AGR–H19). We also determine a small-molecule inhibitor of TRIM63, nifenazone (NIF), as a potential E3 ligase inhibitor. Administration of AGR–H19 or NIF significantly alleviated MD and improved muscle strength and cardiac function in Dmd C3333Y animals in vivo. Additionally, mdx mice subjected to Dmd exon skipping exhibit robust levels of Ub-DMD, which are inhibited following administration of AGR–H19 or NIF. Our findings suggest the importance of lncRNAs and lncRNA-related signalling events in...
inherited genetic muscle disorders and shed light on the therapeutic potential of RNA oligonucleotides as an innovative treatment option for these diseases.

**Results**

**Dystrophin associates with H19.** The C-terminal zinc finger domain (ZNF) of dystrophin assists in the formation of the dystrophin-associated protein complex [2], and the ZNF could potentially serve as an atypical RNA-binding domain [3]. In human and mouse skeletal muscle tissues, crosslinking and immunoprecipitation (CLIP) assays indicated that dystrophin associates with RNA motifs of the lncRNA H19: nucleotides 1954–1974 in human H19 (hH19) and nucleotides 1907–1924 in mouse H19 (mH19) (Fig. 1a,b; Extended Data Fig. 1a and Supplementary Tables 1 and 2). These interactions were confirmed using RNA immunoprecipitation (RIP) assays and RNA fluorescence in situ hybridization (FISH) coupled with immunofluorescence staining (Fig. 1c and Extended Data Fig. 1b,c). The dystrophin C termini (amino acids 3046–3685) exhibited interactions with biotinylated H19 in vitro, but not the other domains of dystrophin or the other components of the dystrophin-associated protein complex (DAPC) that we tested (Extended Data Fig. 1d).

Mouse C2C12 myoblasts with H19 or Dmd knocked out and differentiated into myotubes showed minimally altered Dmd or H19 expression (Extended Data Fig. 1e–h). CLIP rescue assays indicated that Dmd or H19 depletion abolished RNA–DMD complexes (Fig. 1d). Electrophoretic mobility shift assays (EMSA) suggested that RNA, but not DNA, oligonucleotides representing hH19 (nucleotides 1951–1980) associated with dystrophin (Fig. 1e). hH19 RNA, but not androgen receptor 3′ untranslated region RNA or hH19 DNA, served as a competitor (Fig. 1e).

AT-rich motifs play essential roles in mediating interactions between RNA and the ZNF [4]. The H19 RNA motif (nucleotides 1951–1980) contains two AT-rich motifs at nucleotide positions 1954–1957 and 1970–1973 (Fig. 1f). Loss-of-function (LoF) mutations (LoF1, LoF2, LoF3 and LoF4) all exhibited impaired H19–DMD interactions were rescued by the expression of H19 WT but not the LoF mutant (Fig. 1h–j). The dystrophin C termini (amino acids 3046–3685) exhibited interactions with biotinylated H19 in vitro, but not the other domains of dystrophin or the other components of the dystrophin-associated protein complex (DAPC) that we tested (Extended Data Fig. 1d).

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H19 competes with TRIM63 to inhibit the polyUb and protein degradation of dystrophin. Mass spectrometry indicated that in mH19 WT myotubes, dystrophin associates with DAPC components; however, in mH19 knockout (KO) myotubes, mDMD associated with UBA1, UB2G1, TRIM63 and ubiquitin (Supplementary Table 3). In mH19 KO cells, mDMD was modified by K48-linked polyUb at Lys 3577 (Fig. 2a and Extended Data Fig. 1n). We generated a modification-specific antibody targeting human polyubiquitinated DMD (Lys 3584), which is conserved with mDMD Lys 3577, using a double glycine (GG)-modified peptide (referred to as Ub-DMD). A blocking peptide representing Ub-DMD, but not Ub-TPS416, diminished recognition of the Ub-DMD antibody in iPSC-derived SkMCs (Extended Data Fig. 2a). TRIM63 catalysed Ub-DMD in a K48-ubiquitin-dependent manner (Fig. 2b). [35S] methionine pulse–chase assay suggested that H19 depletion leads to a reduced half-life of dystrophin and elevated Ub-DMD, which was restored after the expression of exogenous H19 WT but not LoF mutants (Fig. 2c and Extended Data Fig. 2b–d).

In Dmd-deficient C2C12-differentiated myotubes, we expressed five previously reported non-silent mutations of DMD adjacent to the ubiquitination site (A3421V in BMD1, F3228L in CMD3B4, and C3313Y13, D3335H23 and C3340Y24) that might be involved in interacting with β-dystroglycan25,26. Compared to dystrophin WT, all mutants exhibited impaired interactions with H19, robust Ub-DMD and unaltered expression of H19 (Fig. 2d,e and Extended Data Fig. 2e,f). The presence of hH19 sense transcript, but not anti-sense, inhibited the Ub-DMD of dystrophin WT and mutants (Fig. 2f). WT dystrophin expression in DMD-deficient C2C12-differentiated myotubes rescued dystrophin expression, whereas DMD C3340Y expression resulted in reduced protein detection (Fig. 2g,h and Extended Data Fig. 2g,h). WT H19 expression in DMD C3340Y-expressing C2C12-differentiated myotubes led to significantly increased dystrophin levels compared with cells transfected with blank vectors or the H19 LoF4 mutant (Fig. 2g,h). These observations suggest that overexpression of H19 may restore dystrophin stability in patients with DMD.

TRIM63 and H19 associated with the C-terminal ZNF of dystrophin (Extended Data Figs. 2i and 3a,b). We hypothesized that H19 competes with TRIM63 to inhibit dystrophin degradation. Dystrophin–TRIM63 interactions were reduced in the presence of H19 RNA (Supplementary Fig. 1). The C3340Y mutant exhibited increased binding to TRIM63; however, the presence of H19 decreased this association (Extended Data Fig. 3c). We synthesized RNA mimics representing scramble, H19 WT or the H19 LoF4 mutant (referred to as H19 mut). The H19 mut showed minimal

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**Fig. 1 | H19 interacts and stabilizes dystrophin.** a, CLIP assays using human skeletal muscle tissue samples were visualized by autoradiography (upper) and immunoblotting (IB; lower). DMD-bound RNA (indicated by blue boxes) were subjected to Sanger sequencing. b, Summary of Sanger sequencing of CLIP assay samples. The chromatin sequences corresponding to RNA (negative stranded) bound by DMD are shown. Asterisks indicate the conserved nucleotides between human and mouse. c, RIP assay using indicated antibodies in human skeletal and cardiac muscle. The linc–YY1 interaction was included as a positive control. RT, reverse transcription. Data shown as the mean ± s.e.m., n=3 independent experiments, two-way ANOVA. d, Autoradiography (upper) and immunoblotting (lower) of the CLIP assay in H19- or Dmd-deficient C2C12 differentiated myotubes. Numbers 8 and 10 represent DMD KO, numbers 2 and 13 represent H19 KO. e, EMSA using His-tagged DMD (amino acids (aa) 3046-3685) and (γ-32P)-labelled human H19 RNA (nucleotides (nt) 1951–1980). The unlabelled H19 mRNA/DNA (nt 1951–1980) or androgen receptor (AR) 3′ untranslated region (UTR) RNA served as the competitor. f, Results of the competition binding assay to determine Kd values of the interaction between His-tagged DMD (aa 3046-3685) and biotinylated-hH19 (full length). Unlabelled H19 RNA WT or indicated mutants served as the competitor. Data shown as the mean ± s.e.m., n=3 independent experiments. The sequence of H19 nt 1951–1980 of WT or mutants is shown. g, RIP assay using the indicated antibodies in H19-depleted hiPSC-derived SkMCs (hiPSC-SkMC) expressing H19 WT or indicated mutants. Data shown as the mean ± s.d., n=3 independent experiments, two-way ANOVA. h, Representative images (h) and statistical analysis (i) of DMD staining intensities in H19-depleted hiPSC-derived CMs (hiPSC-CM) or hiPSC-SkMC expressing H19 WT or indicated mutants: DAPI, 4,6-diamidino-2-phenylindole; MyoG, myogenin; Sarc, sarcomeric alpha actin. Scale bars, 50 µm (h). Data shown as the mean ± s.d. (i), n=6 independent experiments, one-way ANOVA. j, Immunoprecipitation (IP) and IB detection of indicated proteins in H19-depleted iPSC-derived SkMCs expressing H19 WT or indicated mutants. α-DG, α-dystroglycan; β-DG, β-dystroglycan; nNOS, neuronal nitric oxide synthase; α-SG, α-sarcoglycan; β-SG, β-sarcoglycan; δ-SG, δ-sarcoglycan; γ-SG, γ-sarcoglycan; α-SNT, α1-syntrophin; β-SNT, β1-syntrophin. P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. Autoradiography and immunoblots are representative of two independent experiments. Statistical data and unprocessed immunoblots are provided as source data.
effect on the secondary structure of H19 RNA mimics (Extended Data Fig. 3d). H19 mimics abolished the interaction between dystrophin C3340Y and TRIM63 (Extended Data Fig. 3e). These results suggest that H19 associates with the ZNF of dystrophin and inhibit TRIM63-dependent Ub-DMD.

The Dmd C3333Y mutant leads to MD. We introduced a single-nucleotide mutation into the mouse Dmd gene ChrX:85,141, 770G>A to achieve a Dmd C3333Y mutant in a C57B/6N genetic background (Fig. 3a and Extended Data Fig. 3g). Male and female Dmd C3333Y animals exhibited unaffected body weight and normal embryonic development (Extended Data Fig. 3h,i). Dmd C3333Y animals showed slightly reduced viability and fertility (Supplementary Tables 4 and 5). By 4 weeks of age, the blood CK concentrations of both male and female Dmd C3333Y animals were elevated compared with WT or heterozygous...
Heterozygous female animals were histologically unaffected compared with WT littermates (Fig. 3b and Extended Data Fig. 3k). By 20 weeks of age, Dmd C3333Y mice displayed multiple areas of Evans blue dye (EBD) uptake in skeletal muscle fibres, which corresponded to acute histopathological features of necrosis (Fig. 4i). Cardiac muscle also showed EBD uptake in Dmd C3333Y mice, which was undetectable in WT littermates (Fig. 4j).

Microfil perfusion indicated that Dmd WT mice exhibit coronary microvessels, which were distributed normally and smoothly tapered in WT mice (Fig. 4k). In contrast, Dmd C3333Y mice displayed numerous areas of pronounced constrictions and as pre- and post-stenotic dilation and microaneurysms (Fig. 4k). Areas with extensive focal vascular lumen narrowing and sparseness of perfusion were observed in Dmd C3333Y mice, but not in WT littermates (Fig. 4k). These results suggest that Dmd C3333Y plays important roles in the onset of myocardial ischaemic lesions.

H19 mimics and NIF inhibit TRIM63-dependent Ub-DMD. Dmd C3333Y skeletal muscle exhibits elevated Ub-DMD and concurrently reduced, but measurable, dystrophin protein levels; both Ub-DMD and dystrophin levels were diminished at the age of 46 weeks (Extended Data Fig. 5a–c). These observations aligned with the reduced, yet detectable, dystrophin levels measured in patients with DMD C3340Y31. We hypothesized that inhibition of Ub-DMD could prolong the half-life of dystrophin and alleviate the symptoms of patients with MD.

Two strategies were considered for potentially inhibiting Ub-DMD: (1) increase DMD–H19 interactions and (2) block the enzymatic activity of the E3 ligase TRIM63. To assess these strategies, we collected iPSC lines derived from a healthy donor and from patients with BMD (Extended Data Fig. 5d,e and Supplementary Table 1). These were further differentiated into SkMCs or CMs, and Ub-DMD was robustly detected in BMD-derived cells (Supplementary Fig. 2a–c). Compared to healthy donors, BMD iPSC-derived SkMCs harboured reduced dystrophin and DMD–H19 interactions (Extended Data Fig. 5a,b). Conversely, TRIM63–dystrophin was increased compared to healthy donors (Extended Data Fig. 5a, Table 1). These were further differentiated into SkMCs or CMs, and Ub-DMD was robustly detected in BMD-derived cells (Supplementary Fig. 2a–c).

Comprehensive lab animal monitoring system (CLAMS) analysis indicated that Dmd C3333Y animals exhibited similar food intake but significantly reduced VO2, VCO2 and energy expenditure compared with WT littermates (Fig. 3b and Extended Data Fig. 4f). Fibrosis and phagocytosis of necrotic muscle tissues were observed, with fat tissues replacing lost muscle tissues (Fig. 3e,f). Both male and female Dmd C3333Y animals showed significantly reduced muscular strength compared with heterozygous or WT littermates, as indicated by gripping strength, hanging time, running distance and time-to-exhaustion experiments (Fig. 3g, Extended Data Fig. 4g and Supplementary Videos 3 and 4). The animals also exhibited decreased kyphotic index values and extensive lung fibrosis upon death, which represents the respiratory failure observed in patients with DMD (Extended Data Fig. 5h,i).

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against representative E3 ligases, including Ring finger E3 ligases (TRIM63, MDM2 (ref. 28), RNF4 (ref. 29), MEX3C (ref. 30) and cIAP1 (ref. 31)), HECT family of E3 ligases (ITCH)32 and U-box E3 ligase (E6AP)33 and found that NIF exhibited effective inhibition against TRIM63 (IC50 value of 1.1 µM) and low inhibition against other E3 ligases (Extended Data Fig. 6e). The Ub-DMD, but not
Fig. 3 | Dmd C3333Y models progressive MD. a, Schematic of the CRISPR-Cas9 method used to generate Dmd C3333Y mice. b, CK concentration comparison of male Dmd C3333Y and Dmd WT mice at indicated ages. Date show the mean±s.d., n=5 animals per experimental group, two-way ANOVA. c, Kaplan–Meier survival analysis of Dmd WT and Dmd C3333Y mice (log rank test). d, Dissection images of the deceased animals indicate the skeleton muscle and respiratory muscle thoroughly calcified, indicated by green arrows, in Dmd C3333Y mice. e, Masson’s trichrome staining and H&E staining of quadriceps (QUA) and diaphragm (DIA) of Dmd WT and Dmd C3333Y mice at the indicated age. f, Statistical analysis of the percentage of central nucleic muscle fibre (upper) and necrotic area of indicated muscle pieces (lower) of Dmd WT and Dmd C3333Y mice at the indicated ages. GAS, gastrocnemius; PEC, pectoralis; TA, tibialis anterior; TRI, triceps. Data shown as the mean±s.d., n=8 animals per experimental group, two-way ANOVA. g, Muscle strength test (upper) and hanging test (lower) of 12-week-old male and female Dmd WT and Dmd C3333Y mice. Data shown as the mean±s.d., n=8 animals per experimental group, unpaired Student’s t-test. h, Energy expenditure measurement of Dmd WT and Dmd C3333Y mice. Data shown as the mean±s.e.m., n=4 animals in each group, two-way ANOVA. P>0.05, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Statistical data are provided as source data.
Fig. 4 | *Dmd* C3333Y mutation causes heart and blood vessel irregularity. a, End-diastolic and end-systolic cine MRI scans from *Dmd* WT and *Dmd* C3333Y mice. Scale bars, 1 mm. b, RVFS (%) and LVFS (%) were measured with ImageJ based on the MRI scans of *Dmd* WT and *Dmd* C3333Y mice at age 24 weeks (n = 5 animals per group). Data shown as the mean ± s.e.m., unpaired Student’s t-test. c, LVEF (upper) and LVFS (lower) of *Dmd* WT (n = 15 animals) and *Dmd* C3333Y mice (n = 18 animals) measured by echocardiography at age 24 weeks. Data shown as the mean ± s.d. d, Representative ECG traces of *Dmd* C3333Y mice, showing sinus bradycardia, sinus arrest and atrioventricular (AV) block. e, Statistical analysis of heart rate (beats per min; b.p.m.), PR interval, QRS duration over time, QT interval and R amplitude plotted from *Dmd* WT and *Dmd* C3333Y mice lead II ECG at 46 weeks old. Data shown as the mean ± s.e.m., unpaired Student’s t-test. f, Masson’s trichrome and H&E staining of cardiac cross-sections of *Dmd* WT and *Dmd* C3333Y mice at 46 weeks old. g-h, Percentage of central nucleic fibre (g) or necrotic area (h) of *Dmd* WT and *Dmd* C3333Y heart. Data shown as the mean ± s.d., n = 7 animals per group, unpaired Student’s t-test. i, Left: front and back views of *Dmd* WT and *Dmd* C3333Y mice 24-h post-EBD injection. Right: statistical analysis of EBD-staining-positive area per muscle of *Dmd* WT and *Dmd* C3333Y mice. Black arrows, the EBD penetrated tissues. Data shown as the mean ± s.d., n = 8 animals per group, two-way ANOVA. j, Representative images of immunofluorescence detection of EBD penetration in cardiac muscle (left) and statistical analysis of EBD-positive area in *Dmd* WT and *Dmd* C3333Y heart (right). Scale bars, 200 µm. k, Transillumination microscope visualization of silicon-rubber-perfused coronary arteries in *Dmd* WT and *Dmd* C3333Y mice. Scale bars, 200 µm. Right: statistical analysis of the number of irregular blood vessels in *Dmd* WT and *Dmd* C3333Y hearts. Black arrows, the irregular blood vessels. Data shown as the mean ± s.d., n = 11 animals per group, unpaired Student’s t-test. ***P < 0.001 and ****P < 0.0001. Statistical data are provided as source data.
Fig. 5 | H19 mimics and NIF attenuate Ub-DMD. a, IP and IB detection of indicated proteins in hiPS-SkMC derived from healthy (H.) donor (GM09503) or patients with BMD (GM04981, GM02298, GM05089 and GM04569). b, RIP assay using indicated antibodies in hiPS-SkMC derived from healthy donor (GM09503) or patients with BMD (GM04981, GM02298, GM05089 and GM04569). Data shown as the mean ± s.d., n = 4 independent experiments, two-way ANOVA. c, In vitro polyUb chain formation using His-tagged DMD (aa 3046–3685), UBA1, UB2G1, TRIM63, ubiquitin and indicated mimics or small-molecule inhibitors. The Ub-DMD process was subjected to Ni-NTA affinity resin pulldown and IB detection using the indicated antibodies. d, Half-life determination of DMD protein in human iPS-CM treated with, vehicle (veh.), H19 mimics or NIF. Data shown as the mean ± s.d., n = 5 independent experiments, two-way ANOVA. e, Representative images of immunofluorescence using indicated antibody (upper) and statistical analysis of DMD staining intensities (lower) of hiPS-SkMC derived from healthy donor or patients with BMD after indicated treatments. Scale bars, 50 µm. Data shown as the mean ± s.d., n = 5 independent experiments, two-way ANOVA. False discovery rate (FDR) > 0.05, *FDR < 0.05, **FDR < 0.01, ***FDR < 0.001 and ****FDR < 0.0001. Immunoblots are representative of two independent experiments. Statistical data and unprocessed immunoblots are provided as source data.
Fig. 6 | AGR–H19 alleviates MD and cardiomyopathy. a, Left: schematic illustration of AGR–H19. Right: scheme of the experimental approach. b, Representative images of H&E staining, Masson’s staining, immunofluorescence of DMD, Ub-DMD (Lys 3357) of QUA and heart tissue H&E staining of Dmd WT or Dmd C3333Y mice treated with AGR–Scr, AGR–H19 or AGR–H19 mut mimics as indicated. Scale bars, 1 mm (specified) or 100 μm (unspecified). c, Percentage of necrotic area of Dmd WT or Dmd C3333Y mice given the indicated AGR–Scr, AGR–H19 or AGR–H19 mut mimics. Data shown as the mean ± s.d., n = 5 animals per group, two-way ANOVA. d, e, Statistical analysis of DMD (d) and Ub-DMD (Lys 3357) (e) staining intensities of QUA muscle of Dmd WT or Dmd C3333Y mice given the indicated AGR–Scr, AGR–H19 or AGR–H19 mut mimics. Data shown as the mean ± s.d., n = 5 animals per group, one-way ANOVA. f, Mouse forelimb muscle strength test of male and female Dmd WT or Dmd C3333Y mice treated with the indicated AGR–Scr, AGR–H19 or AGR–H19 mut mimics. Data shown as the mean ± s.d., n = 8 animals per group, two-way ANOVA. g, Percentage of the fibrotic area in the heart of Dmd WT or Dmd C3333Y mice given the indicated AGR–Scr, AGR–H19 or AGR–H19 mut mimics. Data shown as the mean ± s.d., n = 5 animals in each group, one-way ANOVA. h, i, Ejection fraction percentage (h) or fraction shortening percentage (i) assessment of Dmd WT or Dmd C3333Y mice given the indicated AGR–Scr, AGR–H19 or AGR–H19 mut mimics. Data shown as the mean ± s.d., n = 5 animals in each group, one-way ANOVA. P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. Statistical data are provided as source data.
Fig. 7 | NIF relieves MD and improves skeletal and cardiac muscle function. a, Representative images of H&E staining, Masson’s staining and immunofluorescence of DMD, Ub-DMD (Lys 3584) of QUA and heart tissue H&E staining of Dmd WT or Dmd C3333Y mice treated with vehicle, NIF or nutlin-3. Scale bars, 1 mm (specified) or 100 µm (unspecified). b, Percentage of muscle fibre necrotic area (upper) or muscle fibres with centrally located nuclei (lower) of Dmd WT or Dmd C3333Y mice given vehicle, NIF or nutlin-3. Data shown as the mean±s.d., n = 5 animals per experimental group, two-way ANOVA. c, d, Statistical analysis of DMD (c) and Ub-DMD (Lys 3584) (d) staining intensities of QUA of Dmd WT or Dmd C3333Y mice treated with vehicle, NIF or nutlin-3. Data shown as the mean±s.d., n = 5 animals per experimental group, one-way ANOVA. e, Mouse forelimb muscle strength test of male and female Dmd WT or Dmd C3333Y mice given vehicle, NIF or nutlin-3. Data shown as the mean±s.d., n = 5 animals per experimental group, two-way ANOVA. f, Percentage of fibrotic area in the heart of Dmd WT or Dmd C3333Y mice given vehicle, NIF or nutlin-3. Data shown as the mean±s.d., n = 5 animals per experimental group, one-way ANOVA. g, Ejection fraction percentage (upper) or fraction shortening percentage (lower) assessment of Dmd WT or Dmd C3333Y mice treated with vehicle, NIF or nutlin-3. Data show the mean±s.d., n = 5 animals in each group, one-way ANOVA. P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. Statistical data are provided as source data.
**Fig. 8** | AGR-H19 or NIF enhances DMD protein in combination with exon-skipping therapy.  

a–c, Representative images (a), statistical analysis of DMD (b) and Ub-DMD (c) of immunofluorescence staining of iP-SkMC derived from a healthy donor (GM09503) or a patient with DMD (GM25313) treated with scramble and vehicle or h44AON1 in combination with H19 mimics or NIF, respectively. Scale bars, 50 μm (a). Data shown as the mean ± s.d. (b and c), n = 5 independent experiments, one-way ANOVA.  

d–f, Representative images (d), statistical analysis of DMD (e) and Ub-DMD (f) intensities of immunofluorescence staining of C57BL/10J QUA treated with scramble RNA and vehicle, or mdx QUA treated with scramble, PPMO23 alone or in combination with AGR–H19 or NIF, respectively. Scale bars, 100 μm (d). Data shown as the mean ± s.d. (e and f), n = 5 animals per experimental condition, one-way ANOVA.  

**g**, Forelimb strength of both male and female C57BL/10J mice treated with scramble RNA and vehicle, or mdx mice treated with scramble, PPMO23 alone or in combination with AGR–H19 or NIF, respectively. Forelimb strength was tested and normalized by body weight. Data shown as the mean ± s.d., n = 8 animals per group, two-way ANOVA. *P > 0.05, **P < 0.05, ***P < 0.01, ****P < 0.001 and *****P < 0.0001. Statistical data are provided as source data.
polyUb of MD2-dependent p53 (ref. 3), RNF4-mediated PML (ref. 33), E6AP-catalysed β-arrestin-1/2 (ref. 34), ITCH-dependent DVL2 (ref. 35) or cIAP-1-mediated caspase-3 (ref. 36), was inhibited by NIF (Extended Data Fig. 6f).

Compared to healthy donors, BMD iPSC-derived CMs exhibited a reduced dystrophin half-life (Fig. 5d and Extended Data Fig. 6g). H19 mimics and NIF stabilized dystrophin and attenuated the status of Ub-DMD (Lys3584) in the SkMCs and CMs from iPSCs derived from patients with BMD (Fig. 5e–g, Extended Data Fig. 7a–g and Supplementary Fig. 3a,b). K3584R mutant knock-in in BMD iPSC-derived SkMCs exhibited abolished Ub-DMD, which was minimally affected following AGR–H19 or NIF treatment (Extended Data Fig. 8a–f), thereby mechanistically validating the TRIM63-dependent polyUb of dystrophin.

**AGR–H19 and NIF alleviate MD.** Inspired by peptide-facilitated macromolecular delivery, we applied an AGR-devised peptide to improve the in vivo distribution of H19 mimics in skeletal and cardiac muscles. The AGR Z8 loop associates with low-density lipoprotein receptor-related protein 4 (LRP4) and muscle-specific kinase (MuSK) (ref. 37), leading to the formation of muscle acetylcholine receptor (AChr) clustering (ref. 38). Peptides representing the AGR Z8 loop were conjugated to the 2′-F-C-modified H19 mimics, referred to as AGR–H19 (Fig. 6a and Supplementary Fig. 4). We determined the pharmacokinetics of biotin-labelled H19 and AGR-H19 mimics, and found a detectable amount of AGR–H19 in skeletal and cardiac muscles 3–72 h after dosing (Extended Data Fig. 8g–i and 9a). AGR-tagged RNA mimics showed minimal effects on the formation of AChr clusters, but interacted with LRP4 (Extended Data Fig. 8j).

We applied the AGR–H19 mimics to *Dmd* C3333Y mice from 4 to 16 or 24 weeks of age to determine skeletal or cardiac muscle function, respectively (Fig. 6a). To rule out potential indirect effects, we included AGR–H19 mut as a negative control. AGR–H19 exhibited minimal effects on body weight, liver or kidney function, serum IGF1 and IGF2 levels or expression status of microRNAs we tested in *Dmd* C3333Y animals (Extended Data Table 8). Although *Dmd* C3333Y animals treated with AGR–H19 exhibited similar blood CK levels compared with animals treated with a scramble mimic, skeletal muscles of C3333Y mice exhibited reduced necrotic areas and percentage of central nuclei-containing fibres (Fig. 6b,c and Extended Data Fig. 9j,k). Animals treated with AGR–H19 exhibited elevated dystrophin and reduced Ub-DMD levels in sarcolemma, as well as improved strength (Fig. 6b–f). *Dmd* C3333Y hearts also showed reduced fibrotic areas and increased LVEF and LVFS (Fig. 6b,g–i).

Treatment with NIF minimally affected body weight, blood CK levels and liver and kidney function (Fig. 7a, Extended Data Fig. 9l,m and Supplementary Table 8). NIF, but not nutlin-3, significantly reduced the necrotic area and central nuclear fibres of *Dmd* C3333Y animals, and restored dystrophin and decreased Ub-DMD levels (Fig. 7a–d). Administration of NIF significantly improved the strength of *Dmd* C3333Y animals (Fig. 7c). Furthermore, *Dmd* C3333Y hearts showed reduced fibrotic areas and improved function after NIF treatment (Fig. 7f,g).

Antisense oligonucleotide-mediated exon skipping results in elevated dystrophin status in both animals and clinical trials (ref. 39). Phosphorodiamidate morpholino antisense oligonucleotides (PMOs) have been shown to be effective in improving strength and walking distances in patients with DMD (ref. 40). However, the induction of exon skipping leads to the development of a BMD-like phenotype, in which the expressed dystrophin might exhibit increased Ub-DMD and protein degradation. iPSCs derived from patients with DMD subjected to h44AON1-mediated exon skipping showed partially restored protein expression of dystrophin, with robust Ub-DMD status (Fig. 8a–c). H19 mimics interacted with truncated dystrophin (Ex45Del) and inhibited the recruitment of TRIM63 to truncated dystrophin (Extended Data Fig. 10a). H19 mimics and NIF treatment significantly reduced the Ub-DMD status and improved the protein level of dystrophin in a K3584-dependent manner (Fig. 8a–c and Extended Data Fig. 10b–f).

*mdx* mice given a PMO conjugated to a cell-penetrating peptide (PPM023), as previously reported (ref. 41), exhibited partially restored expression of dystrophin in skeletal muscle and robust polyUb (Fig. 8d–f). Co-treatment with AGR–H19 or NIF significantly increased the protein levels of dystrophin and reduced the status of Ub-DMD and animal strength in PPM023-treated *mdx* mice (Fig. 8d–g).

Taken together, our research findings suggest that dystrophin is subjected to TRIM63-mediated polyUb and protein degradation (Supplementary Fig. 5), thereby illustrating one of the mechanisms of MD. Administration of AGR–H19 or NIF may alleviate the progression of MD, thus providing a promising therapeutic strategy for BMD and certain patients with DMD both alone or in combination with exon-skipping therapy strategies.

**Discussion**

The low or undetectable levels of dystrophin protein in BMD and in certain patients with DMD harbouring point mutations and in-frame deletions might be partially due to the shortened half-life of dystrophin, which is mediated by TRIM63-dependent polyUb. H19 competes with TRIM63 in interacting with the ZNF of dystrophin to hinder polyUb and degradation of the protein. Genetic evidence suggests that *Dmd* C3333Y animals exhibit progressive MD phenotypes that model human patients with DMD harbouring the C3340Y mutation. BMD hiPSC-derived SkMCs and CMs exhibited elevated Ub-DMD, which suggests that a cohort of patients with BMD exhibit an increased incidence of dystrophin polyUb and expedient dystrophin turnover. An antibody targeting Ub-DMD could potentially identify patients with BMD who could benefit from a treatment that inhibits dystrophin degradation. Administration of AGR–H19 and NIF provides a potential complementary therapeutic to current exon-skipping technologies to stabilize dystrophin protein following PMO treatment to improve the efficacy of slowing the progression of MD.

The reduced lifespan of C3333Y mice might due to dystrophy of the diaphragm, cardiac muscles and smooth muscles, which could be less affected in *mdx* mice (ref. 42). Microdystrophins, including dp116 and dp71, could be degraded in C3333Y mice. Transgenic expression of dp116 extends the lifespan of animals lacking both dystrophin and utrophin (ref. 43). The adeno-associated virus (AAV)-mediated expression of dp116 in *mdx* tibialis anterior muscle induced rapid loss (ref. 44). A possible explanation of this effect is that the AAV-expressed dp116 might also be subjected to TRIM63-mediated polyUb and interstitial protein degradation, which could be attenuated by AGR–H19 or NIF.

ZNF has been established as a typical RNA-binding domain and plays important roles in mediating the interactions between dystrophin and β-dystroglycan (ref. 45). C3340 serves as one of the conserved residues of typical C2-H2 zinc fingers (ref. 46). It is possible that C3340Y impairs the docking of zinc ions to cause potential conformational changes in this domain. In-frame deletion of *DMD* in patients with BMD leads to a truncated form of dystrophin (ref. 47). Given the binding of amino and C termini of dystrophin to the cytoskeleton (ref. 48), truncated dystrophin experiences potential conformational changes in the ZNF. For patients with DMD, exon-skipping technologies convert out-of-frame deletions into in-frame deletions (ref. 49), thereby producing BMD-like phenotypes, but the newly translated yet truncated form of dystrophin is subjected to fast turnover. To overcome these impairments, AGR–H19 competes with TRIM63 in interacting with mutated/truncated dystrophin, and NIF inhibits enzymatic activities of TRIM63.
H19 is upregulated in multiple tumour types and serves as a microRNA precursor; however, the 30-nucleotide-long H19 mimics we developed did not affect the function of these microRNAs. Administration of the H19 mimics minimally affected the RNA expression status of H19, the RNA and protein levels of IGFI1 and IGF2 or the mRNA levels of DMD. Hence, our data suggest that the administration of AGR–H19 leads to minimal risk of tumorigenesis.

The specific expression of TRIM63 in skeletal and cardiac muscle makes this protein a promising therapeutic target for MD. NIF effectively inhibited the TRIM63-dependent protein degradation of dystrophin and possibly other cellular targets. NIF has been used as an analgesic for certain rheumatic disorders, and the mild side effects ensure safe long-term application. Hence, our data suggests that NIF is a promising candidate for alleviating the clinical impact associated with MDs.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-020-00595-5.

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Methods
In vivo murine models and treatment. All animal-based research was conducted according to the guidelines and requirements set forth by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals, the US Department of Health and Human Services Guide for Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 as amended by the Institutional Animal Care and Use Committee of the University of Texas MD Anderson Cancer Center. The study was compliant with all relevant ethical regulations regarding animal research. Using a CRISPR-Cas9 Extreme Genome Editing System (EGE Biotechnology), we knocked-in a single-nucleotide mutation into the mDmd gene (ChrX:85,141,770G>A in C57BL/6N mice) to achieve a mouse DmdC3333Y mutant. Two independent pronucleus injections and six founder mice were initially achieved, and all the conducted studies were based on the offspring of the six founder mice; we included Dmd WT littermates as a control. StepOne Software (ThermoFisher) was used for data acquisition for TaqMan genotyping PCR. The blood CK concentration was determined at 4, 12, 24 and 28 weeks of age. The survival time of the mice was recorded as the time of natural death or when euthanasia was requested by the veterinarian due to weakness or moribund status. To obtain unbiased and reliable results, all the mice of the same age and the same gender were randomly grouped and housed in the same housing room. All animals were housed with a 12-h light–12-h dark cycle in the animal facility, with free access to water and food. All of the mice were housed at temperatures of 65–75°F (18–23°C) with 40–60% humidity.

For the AGR–H19 treatment, AGR–scramble (AGR-Scr), AGR–H19 or AGR–H19 mut was applied (10 mg per kg, subcutaneously, every 3 days) starting from 4 weeks of age (4–7 weeks old) or 24 weeks of age (16–19 weeks old). For the NIF treatment, NIF or nutilin-3 was applied (10 mg per kg intraperitoneally, daily) starting from 4 weeks of age until 16 or 24 weeks of age. The control group was injected an equal volume of vehicle. Both male and female Dmd WT and DmdC3333Y mice were included in the experimental settings. The general health, grooming and behaviour of all animals were monitored daily, and injection sites were checked for signs of redness or oedema.

Human tissues and hiPSCs. De-identified fresh frozen human skeletal muscle tissues were purchased from ProteoGenex. The study protocol was approved by the Institutional Review Board of the MD Anderson Cancer Center, University of Texas. Clinical information is summarized in Supplementary Table 1. All hiPSC studies were approved by the HEIP Stem Cell committee of the University of Texas, MD Anderson Cancer Center. De-identified human donor fibroblasts cells from patients with BMD (GM04569, GM05089, GM02298 and GM04981) and healthy donor fibroblasts cells (GM09503) were obtained from the Coriell Institute and reprogrammed to iPSCs at the Human Stem Cell Core ( Baylor College of Medicine). The human DMD donor iPSCs (GM25313) were obtained from the Coriell Institute. Clinical information is summarized in Supplementary Table 1. The iPSCs were cultured on plates coated with hESC-Qualified Matrigel (Corning) and maintained in feeder-free mESR 1 medium (Stemcell Technologies). The pluripotency of these iPSCs were verified using a Human Pluripotent Stem Cell Functional Identification kit (RBD Systems, SC027). The antibodies used and dilution information are listed in Supplementary Table 1. hiPSCs were differentiated into CMs and SKMs using a STEMDiff Cardiomyocyte Differentiation kit (Stemcell Technologies) and a previously described skeletal muscle differentiation protocol 6.

Exon-skipping therapies combined with H19 mimics or NIF treatment. mdx (C57BL/10ScSn-mdx+/-) mice and WT C57BL/10J mice were obtained from The Jackson Laboratory. The PMO against exon 23 of the DMD gene (PMO023) was synthesized and conjugated to the cell-penetrating peptide (RXXRRRXXRXXRXX) (Gene-Tools). Male mdx mice were given PMO023 (15 mg per kg intravenously, twice a week) from 4 weeks of age and were also given AGR–H19 (10 mg per kg subcutaneously, every 3 days) or NIF (10 mg per kg intraperitoneally, daily), and the experiment was terminated at 24 weeks of age. Male WT C57BL/10J mice of same age were included as a control group. The human antisense oligonucleotide (AON) against exon 44 (hA44AON1) of the human DMD gene was synthesized using phosphorothioate-esterified (16 mer) RNA oligonucleotides (Spring Bioscience Inc., USA) and applied to hPSC-derived SKMs and CMs, with scramble RNA delivery as the negative control. H19 mimics and NIF was used to treat the iPSC-derived SkMCs and CMs together with hA44AON1, and vehicle was used as the negative control. All cells were collected 7 days post-treatment. Oligonucleotide sequences are provided in Supplementary Table 9.

Dystrophin protein half-life detection by pulse–chase assay. The half-life of dystrophin protein (DMD) was evaluated by pulse–chase assays. hiPSC-derived CMs were dissociated and passed into 6-well plates at a density of 3 × 10^4 cells per well in a CM maintenance medium (Stemcell Technologies) on day 14 of CM differentiation. After 24 h, the hiPSC-derived CMs from the healthy donor (GM09503) were induced with 1 µg/ml doxycycline to KO H19 and transfected with MS2-tagged WT or mutant H19. At 48 h after passing, the hiPSC-derived CMs were treated with 0.1 µCi [35S] methionine (Perkin-Elmer) in methionine-free DMEM (Thermo Fisher Scientific) with 1% dialysed bovine serum for 48 h. After [35S]-methionine treatment, the medium was then changed to a CM maintenance medium. The cells were collected at varying chase points (72 h after replacing the medium was counted as day 0 of half-life detection), and the cell lysates were subjected for the immunoprecipitation experiment. The used and dilution information are listed in Supplementary Table 10. Immunoprecipitates were analysed by autoradiography. The autoradiography signals corresponding to dystrophin protein (~430 kDa) were quantified using ImageJ software. Protein levels at varying chase points were normalized to day 0 intensity of dystrophin protein and expressed as relative percentage integrated intensity.

Pharmacokinetic studies of RNA mimics. The RNA mimics were synthesized by Bio synthesis Inc. (the oligonucleotide sequences are listed in the Supplementary Table 9). Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) was used as the linker connecting the peptide and RNA oligonucleotide. The pharmacokinetics of the H19 mimics was monitored by determining the biotinylated H19 mimics or AGR–H19 (10 mg per kg) were subcutaneously or intraperitoneally injected into WT C57BL/6J mice. The skeletal muscles, cardiac muscles, livers, kidneys and lungs of animals at 3, 12, 24, 48 and 72 h post-injection were collected (n = 5 animals per time point). Tissues were subjected to immunohistochemistry using anti-smooth muscle actin antibody and small RNA isolation as per the manufacturer’s instructions using miCURY RNA isolation kits (Quagen). Using a dot blot, we titrated the biotin-labelled H19 mimic or AGR–H19 mimics using twofold dilutions ranging from 1 µg to 1 µg. We also plotted the RNA extracted from skeletal muscle, cardiac muscle, liver and kidney on semi-logarithmically indicated timepoints. The AGR–H19 mimics were detected using streptavidin–horseradish peroxidase (HRP). By comparing the blot densities, we calculated the concentrations of H19 mimics and AGR–H19 as ng g−1 tissue. Pharmacokinetic parameters were determined by nonlinear regression analysis.

Generation of knock-in iPSC lines. Single guide RNAs (sgRNAs) were designed to target upstream of exon 75 of the DMD gene located in chromosome X. For the targeting site, candidate guide RNAs were designed using the CRISPR design tool (http://www.sanger.ac.uk/). Guide RNAs were screened for on-target activity using a Universal CRISPR Activity assay (UCA). UCA, a sgRNA activity detection system developed by Biocytogen, is simpler and more sensitive than the MSDa assay. To minimize random integrations, we employed a circular donor vector. The targeting vector contained a PuroDeltalk cassette and two homology arms of left (1,428 bp), introducing a point mutation with a single base substitution (c.10751A>G), and right (1,266 bp) each was used as a template to repair the double-stranded breaks generated by Cas9/gRNA. sgRNA1 and the targeting vector were electroporated into the iPSC line. For electroporation, 500 µl of cell suspension, 7.5 µg gRNA plasmid, 7.5 µg spCas9 plasmid and 10 µg DNA template plasmid were mixed in a 4-mm cuvette (Bio-Rad) and immediately electroporated with a Bio-Rad Gene Pulser. Electroporation parameters were set at 250 V, 200 µF and infinite resistance. After drug-resistance selection (puromycin, 2 µg ml−1), 96 resistant single-cell-derived colonies were obtained from each parental cell line and expanded. Six homozygous clones for each parental cell line were obtained by further genotyping characterization and PCR product sequencing. The colonies harbouring the resistance cassette were further subjected to Cre-recombinase-mediated removal of the resistance cassette as previously described 7. The pCAG-CRE, expressing the Cre recombinase gene, was obtained from Addgene. The primers for genotyping are provided in Supplementary Table 9. The primer used for each genotyping are indicated in the corresponding figure legend.

CRISPR–Cas9-mediated gene editing. H19 and Dmd KO mouse cell lines and hiPSC-derived CMs and hiPSC-derived SKMs were generated using the CRISPR–Cas9 genome editing system. 293 FT cells were obtained from ThermoFisher (R70007). The gRNA sequence is listed in Supplementary Table 9.

Cell and tissue immunofluorescence staining and AChR clustering. The myoblast-cell-derived hiPSCs were passaged into 24-well plates, which were placed in 24-well plates with coated with collagen-laminin and rhodamine-conjugated phalloidin (ThermoFisher, R71206). Myoblasts reached confluence after being maintained in the skeletal myoblast medium for 4–6 days. H19 KO lentiviruses was induced followed by transfection with lentivirus carrying WT H19 or mutants. The medium was switched to a myotube medium 24 h after transfection. After culturing in the myotube medium for 4 days, the skeletal myotubes were fixed with 4% paraformaldehyde solution. AChR clustering was performed as previously described 8. The images were visualized as 3D stacks. They were imported into a Zeiss Axioskop2 plus microscope. AxioVision software v.4.8.2.0 (Carl Zeiss) was used to acquire microscopy images. All immunostained slides were scanned on an APERIO Scan Scope XT (Leica Biosystems) for quantification by digital image analysis. The quantification of immunofluorescence staining density was performed using Image-Pro plus (Media Cybernetics) and calculated based on the average staining intensity and the percentage of positively stained cells.

CLIP assay. CLIP assays were performed as previously described 9. After immunoprecipitation using antibodies targeting DMD (Abcam, ab15277), the RNA–protein complexes were digested under the + RNase condition or + + +
The mice used were fasted for 4–6 h, anaesthetised with isoflurane, and blood was collected via cardiac puncture. Unless otherwise indicated, mice were weighed and genotyped after collection of blood. 10 male or female mice were used as a group. One male was housed with one female mouse, and there were no mice that were purchased. In the control group, one male was housed with one female mouse, and there were no mice that were purchased. All animals were kept in a 12:12 h light:dark cycle with free access to food and water. The experiments were performed in accordance with the guidelines of the Animal Care and Use Committee of Nanning Medical University. Approval was obtained from the Institutional Animal Care and Use Committee of Nanning Medical University. All efforts were made to minimize suffering and to reduce the number of animals used.

**Determination of $K_d$ values using the alpha assay.** Alpha binding assays were used to quantitatively assess the interaction between H19 and DMD using biotinylated H19 and His-tagged DMD C termini (amino acids 3046–3685) as the donor and acceptor pair. The dissociation constant ($K_d$) was determined via a competition experiment in which WT H19 or four potential LoF mutations (LoF1, LoF2, LoF3 and LoF4) were titrated in 10-fold dilution) was assayed in H19-deficient cells. MS2-tagged H19 was immunoprecipitated, and MS2-tagged H19-associated DMD protein was detected by immunoblotting. The antibodies used and dilution information are listed in Table 10.

**Screen of small-molecule inhibitors targeting TRIM63.** The small-molecule inhibitor screen was performed as previously described65 using Ub-DMD (Lys3584) antibody. A validated hit (2,027 bioactive compounds, Selleckchem) was tested. The polyUb formation of DMD was detected by incubation with Ub-DMD (Lys3584) antibody. The background-subtracted average absorbance of each tested compounds was normalized to dimethylsulfoxide (DMSO)-treated samples and the fold-change was plotted.
and weaned mouse genotype and gender numbers and ratios were compared to expected Mendelian ratios.

Transmission electron microscopy. Samples were fixed with a solution containing 3% glutaraldehyde plus 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3, then washed in 0.1 M sodium cacodylate buffer and treated with 0.1% Millipore-filtered cacodylate buffered tannic acid, post-fixed with 1% buffered osmium, and stained with 1% Millipore-filtered uranyl acetate. Ultrathin sections were cut using a Leica Ultracut microtome (Leica), stained with uranyl acetate and lead citrate in Leica EM Stainer, and examined in a JEM 1010 transmission electron microscope (JEOL) at an accelerating voltage of 80kV. Digital images were obtained using an AMT Imaging System (Advanced Microscopy Techniques).

Blood biochemistry. Serum was collected by tail bleeding or cardiac puncture after the mice were fasted for 4–6h, and CK, creatinine and BUN values were determined by enzymatic assays using commercial kits (BioAssay Systems). Alkaline phosphatase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total bilirubin and total protein levels were measured with the help of the Clinical Pathology Workup at the MD Anderson Cancer Center.

Plasmid construction, siRNAs, transfection and lentiviral transduction. H19 DNA sequences were synthesized by GenScript and cloned into pGEM-3Z vector (Promega) for in vitro transfection in the pCDNA3.1 (+) vector (Life technologies) or pMS2 vector for mammalian expression. Full-length human or mouse DMD DNA sequence was obtained from the MDACC short hairpin RNA and ORFeome Core and Addgene. Gateway pET-DEST42 vector (Invitrogen) was used for the prokaryotic expression of human DMD C termini. Mammalian full-length DMD, ZNF of DMD or H19 WT and mutant vectors were constructed by subcloning the corresponding gene sequences into the SFB-tagged expression vector (provided by J. Chen, MD Anderson Cancer Center) using the Gateway system (Life technologies). All single-point and deletion mutations were generated using a QuikChange Lightning Site-Directed Mutagenesis kit (Agilent Technologies). Scramble short interfering RNA (siRNA) and siRNA targeting TRIM63 were obtained from Santa Cruz Biotechnology. Plasmid transfections were performed using Lipofectamine 3000 (Life Technologies) or electroporation using a 4D-Nucleofector System (Lonza) according to the manufacturer’s instructions. Recombinant DMD WT and mutants were expressed in the Escherichia coli strain BL21-CodonPlus (DE3)-RIPL (Agilent Technologies) and purified using a HisPur Cobalt Resin kit (Thermo Scientific).

Statistics and reproducibility. The study was set up to use three to eight samples/ repeats per experiment/group/condition to detect a twofold difference with 80% power and at the significance level of 0.05 by one-sided or two-sided tests for significant studies. Immunofluorescence staining experiments were independently repeated for three to five times or using five to eight animals per experimental group. RNA FISH coupled with immunofluorescence staining were performed three times independently. Immunoblotting detection was repeated independently two times. CLIP assays and EMSAs were repeated independently two times. Transmission electron microscope data represent independent replicates using five animals per group. Histological staining data represent independent replicates using five to eight animals per group. DNA agarose gel data represent two independent experiments. Results are reported as the mean ± standard error of the mean (s.e.m.) or standard deviation (s.d.) of at least three independent experiments, as indicated in the figure legends. Exact n values are indicated in the corresponding figure legend. Statistical analyses were performed using GraphPad Prism 8 software using post-hoc Tukey testing. Comparisons were analysed by unpaired Student’s t-test, one-way analysis of variance (ANOVA) or two-way ANOVA tests as indicated in the corresponding figure legends. P values are as follows and indicated in individual figures: not significant (NS), P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. For survival analysis, Kaplan–Meier survival curves were compared using the log-rank test.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Mass spectrometry data to identify DMD-binding proteins and post-translational modifications have been deposited in ProteomeXchange with the primary accession code PXD020566 (ftp://ftp.pride.ebi.ac.uk/pride/data/archive/2020/08/PXD020566). All other data supporting the findings of this study are available from the corresponding authors upon reasonable request. Source data are provided with this paper.

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Author contributions
L.Y. and C.L. conceived the project and designed the experiments. Y.Z. executed experimental design and data interpretation. S.D.E. assisted with manuscript drafting. L.Y. and C.L. executed the mass spectrometry analysis. CLIP assays were performed by L.Y. The hiPSCs were generated by J.I.K. and P.Z., and hiPSC culture was performed by Y.Z. with assistance from L. Huang, Y.X., C.S., Z. Zhao, J.W. and R.D. J.C., M.-C. -A.F., A.F. and R.D. contributed to experimental design and data interpretation. S.D.E. assisted with manuscript drafting. Z.Y., L.Y. and C.L. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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**Extended Data Fig. 1 | H19 interacts with dystrophin.** a, CLIP assays using mouse skeletal muscle tissues, followed by autoradiography. Protein-bound RNA (blue box) were extracted for Sanger sequencing. b, RIP assay using mouse skeletal muscle tissue. Mean values ± SD, n = 3 independent experiments, two-way ANOVA. c, Representative images of immune RNA Fluorescence In Situ Hybridization in mouse skeletal and cardiac muscle tissues. Data represents three independent experiments. Scale bars, 50 μm. d, Streptavidin pull-down assay using indicated recombinant protein and biotinylated-H19, followed by immunoblotting (IB). Streptavidin (Strep.)-HRP indicated the presence of biotinylated-H19 transcripts. e, Detection of H19 depletion in C2C12 cells. f, RT-qPCR detection of indicated genes in C2C12 H19-proficient or -KO cell line. Mean values ± SD, n = 4 independent experiments, one-way ANOVA. g and h, RT-qPCR detection (g) or IB detection (h) in Dmd-proficient or -KO C2C12 cell lines. Mean values ± SD (g), n = 4 independent experiments, one-way ANOVA. i, H19 Copy number determination in IPS-derived cardiomyocytes (IPS-CMs) using qPCR. Mean values ± SD, n = 5 independent experiments, one-way ANOVA. j, Representative image of immunofluorescence staining (IF) of IPS-SkMCs and cardiomyocytes (IPS-CMs) derived from healthy donor (GM09503), upon H19 depletion. Scale bars, 50 μm. k and l, IB detection of indicated proteins (k) or RIP assay (l) in H19-proficient or deficient IPS-SkMCs expressing indicated expression vectors. Mean values ± SD (l), n = 3 independent experiments, two-way ANOVA. m, H19 RNA copy number determination in H19-proficient or deficient IPS-CMs expressing indicated expression vectors. Mean values ± SD, n = 6 independent experiments, one-way ANOVA. n, Annotated MS/MS spectrum assigned to the dystrophin peptide with ubiquitin modification at Lys 3577. No significance [n.s.], p > 0.05, * , p < 0.05, **, p < 0.01, ***, p < 0.001. Autoradiography and immunoblots are representative of two independent experiments. Statistical source data and unprocessed immunoblots are provided as Source Data Extended Data Fig. 1.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | H19 antagonizes poly-ubiquitination and protein degradation of DMD. a, Blocking Peptide Competition Assay using cell lysates extracted from H19-proficient, or -deficient iPS-SkMCs and blocking peptides of Scramble, TPSN, Ub-TPSN, DMD and Ub-DMD as shown, followed with IB detection using indicated antibodies. MG132 was used as proteasome inhibitor. b, Autoradiography of immunoprecipitated dystrophin in H19-proficient or -deficient iPS-CMs stably expressing MS2-tagged H19 WT or indicated mutants followed by L-[35S]-Methionine pulse-chase. Input was subjected to IB detection using indicated antibody. c, IB detection of indicated proteins in H19-proficient or deficient hiPS-SkMCs expressing MS2-tagged H19 WT or indicated mutants. d, IB detection of indicated proteins in H19-proficient or deficient C2C12 differentiated myotubes expressing DMD WT or the indicated mutants with PS-341. e, IB detection of DMD in Dmd-proficient or deficient C2C12 differentiated myotubes expressing DMD WT or the indicated mutants. f, RT-qPCR detection of H19 relative expression level in Dmd-proficient or -deficient C2C12-differentiated myotubes expressing DMD WT or indicated mutants. Mean values±SD, n = 5 independent experiments, one-way ANOVA.

g, and h, RT-qPCR detection of Dmd (g) and H19 (h) relative expression level in Dmd-proficient or –deficient C2C12 expressing DMD or H19 WT or indicated mutants. Mean values±SD, n = 4 independent experiments, two-way ANOVA.
i, IB detection using indicated antibodies of Ni-NTA pulldown using recombinant proteins as indicated. No significance [n.s.], p > 0.05, *, p < 0.05, **, p < 0.01, ***, p < 0.001, and ****, p < 0.0001. Immunoblots are representative of two independent experiments. Statistical source data and unprocessed immunoblots are provided as Source Data Extended Data Fig. 2.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | H19 competes with TRIM63 in interacting with dystrophin. a, Immunoprecipitation followed with IB detection using indicated antibodies of hiPS-SkMCs derived from healthy donor (GM09503) expressing DMD WT or indicated mutations. b, RIP assay and RT-QPCR detection of indicated genes of hiPS-SkMC cells derived from healthy donor (GM09503) expressing DMD WT or indicated mutations. Mean values±SD, n = 3 independent experiments, two-way ANOVA. c, IB detection using indicated antibodies of indicated recombinant proteins in the presence of biotinylated H19 RNA transcript. The LINK-A RNA transcript were included as negative control. d, Schematic illustration of predict secondary structure of Scr (scramble RNA) mimic, H19 RNA mimic and H19 mimic carrying mutation (H19 mut). e, IB detection using indicated antibodies of indicated recombinant proteins in the presence of biotinylated H19 RNA transcript. The LINK-A RNA transcript were included as negative control. f and g, Representative images of genotyping (f) and Sanger sequencing analysis (g) of Dmd in Dmd WT, Dmd C3333Y and mdx mice. h and i, Body weight measurement of female (h) or male (i) Dmd WT, Dmd WT/C3333Y and Dmd C3333Y/C3333Y mice. Mean values±SD, n = 5 mice in each group, two-way ANOVA. j, H&E staining representative images of the lung, liver, kidney and spleen tissues in Dmd WT and Dmd C3333Y mice. Scale bars, 100 μm. Data represent independent replicates using 8 animals per group. k, Creatine kinase (CK) concentration in female Dmd WT, Dmd WT/C3333Y and Dmd C3333Y/C3333Y mice serum was tested of 4, 12 and 24-week-old, respectively. Mean values±SD, n = 5 mice in each group, two-way ANOVA. No significance [n.s.], p > 0.05, *, p < 0.05, **, p < 0.01, ***, p < 0.001, and ****, p < 0.0001. Immunoblots are representative of two independent experiments. Statistical source data and unprocessed immunoblots are provided as Source Data Extended Data Fig. 3.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Dmd C3333Y mouse models muscular dystrophy. a, RT-qPCR detection of indicated genes in indicated female mice. Mean values±SD, n=5 mice per group, two-way ANOVA. b, Mouse cytokine array assay using the serum of indicated mice. Data represent independent replicates using 3 animals per group. Serum concentration of indicated cytokines in indicated mice. Mean values±SEM, n=3 (c) or 5 (d) mice per group, unpaired student’s t-test. e, H&E staining representative images of Gastrocnemius, Quadriceps, or Tibialis Anterior in indicated female mice at age of 12 weeks. Scale bars, 100 μm. Data represent independent replicates using 8 animals per group. f, Transmission electron microscope representative images of tibialis anterior (TA) and cardiac muscles. Scale bars, 500 nm. Data represent independent replicates using 5 animals per group. g, Running distance and exhaustion time of indicated mice. Mean values±SD, Female: n=8, 15, 13; Male: n=7, 6, one-way ANOVA. h, Representative images of micro-CT of mouse spine (left) and kyphotic index (right) of male indicated mice. Mean values±SEM, n=6 per group unpaired student’s t-test. i, H&E staining representative images of 46-week-old indicated lung. Scale bars, 1 mm or 100 μm. Data represent independent replicates using 8 animals per group. j, Food intake of male indicated mice. Mean values±SD, n=4 animals in each group, two-way ANOVA. k, VO2 (top) and VCO2 (bottom) consumption of male indicated mice. Mean values±SEM, n=6 animals in each group, two-way ANOVA. l and m, Urinary albumin to creatinine ratio (ACR), blood urea nitrogen (BUN) and serum creatinine measurement of female (left) and male (right) indicated mice. Mean values±SD, Female: n=8, 9; Male: n=8, 6 for WT and C3333Y, respectively, unpaired student’s t-test. No significance [n.s.], p > 0.05, *, p < 0.05, **, p < 0.01, ***, p < 0.001, and ****, p < 0.0001. Statistical source data are provided as Source Data Extended Data Fig. 4.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Dmd C3333Y mutation facilitates poly-ubiquitination and protein degradation of DMD.  

a, IF staining representative images using indicated antibodies in QUA (quadriceps) of Dmd C3333Y and mdx mice at indicated age. Scale bars, 100 μm. Data represent independent replicates using 5 animals per group.  

b, c, IF staining representative images (b) and staining intensity statistics (c) in QUA using DMD and Ub-DMD (Lys3584) antibodies of Dmd WT or Dmd C3333Y mice at indicated age. Scale bars (left), 100 μm. Mean values±SD (right), n = 8 mice in each group, two-way ANOVA.  

d, Colonies karyotyping representative images of hiPS cells derived from human fibroblast cells of indicated donors. Healthy donor: GM09503; BMD patients: GM04981, GM02298, GM05089, GM04569. Data represent independent replicates using 3 clones per cell line.  

e, Flow cytometer verification of human fibroblast cells derived hiPS cells by using OCT4 antibody. Data represent independent replicates using 3 clones per cell line. ****, p < 0.0001. Statistical source data are provided as Source Data Extended Data Fig. 5.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | H19 RNA mimics and Nifenazone promote the protein stability of dystrophin.  
a, IP and IB detection using indicated antibodies in healthy or BMD iP-SkMCs.  
b, IP and IB detection using indicated antibodies in healthy or BMD iP-SkMCs treated with H19 mimics, wild type or mut.  
c, Log of TRIM63 expression profile in different human tissues.  
d, Heat map of sandwich ELISA using His6-TUBE and Ub-DMD (lys3584) antibody for screening customized compound library and the generation of the Ub-DMD was detected by OD450. Log2 of relative fold change of polyUb chain formation activity was shown.  
e, Competition curve determination of IC50 value of NIF on the enzymatic activity of indicated E3 ligases. The IC50 values of NIF against each E3 ligase are shown. Mean values ± SD; n = 3 independent experiments.  
f, Left: IB detection using indicated antibodies of hiPS-SkMCs upon vehicle (Veh.) or NIF treatment. Right: list of E3 ligase and known cellular substrates as shown.  
g, Autoradiography of immunoprecipitated dystrophin in iP-CMs derived from healthy donor or BMD patient stably expressing MS2-tagged H19 or treated with vehicle or Nifenazon (10 µM) followed by L-[35S]-Methionine pulse-chase. Input was subjected to IB detection using indicated antibody. Immunoblots are representative of two independent experiments. Statistical source data and unprocessed immunoblots are provided as Source Data Extended Data Fig. 6.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | H19 and Nifenazone extend the half-life of DMD protein. a, Representative images of IF staining using indicated antibody (top) and statistical analysis of DMD staining intensities (bottom) of hiPS-CMs derived from healthy donor or BMD patients, upon transfection of Scramble (Scr) mimic, H19 mimic or treatment Nifenazone (10 μM). Scale bars, 50 μm. Mean values±SD, n=5 independent experiments, two-way ANOVA. b, Representative images of IF staining using indicated antibody (top) and statistical analysis of Ub-DMD (Lys3584) staining intensities (bottom) of hiPS-CMs derived from healthy donor or BMD patients, upon transfection of Scramble (Scr) mimic, H19 mimic or treatment Nifenazone (10 μM). Scale bars, 50 μm. Mean values±SD, n=5 independent experiments, two-way ANOVA. c, IP using DMD antibody followed by IB detection using indicated antibodies in hiPS-SkMCs from healthy donor or BMD patients with the indicated treatments. d–g, RT-QCPR detection of indicated genes of hiPS-SkMCs derived from healthy donor or BMD patients, upon transfection of Scramble (Scr) mimic, H19 mimic or treatment Nifenazone (10 μM). Mean values±SD, n=5 independent experiments, two-way ANOVA. No significance [n.s.], p > 0.05, *, p < 0.05, **, p < 0.01, ***, p < 0.001, ****, p < 0.0001. Immunoblots are representative of two independent experiments. Statistical source data and unprocessed immunoblots are provided as Source Data Extended Data Fig. 7.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | DMD K3584R resists TRIM63-dependent polyUb and protein degradation. a, Graphic illustration of the strategies used to generate knockin cell lines derived from hiPS cells. b, EGFP expression of hiPS cells upon electroporation. Scale bars, 50 µm. Data represent 3 independent experiments. c, DNA agarose gel detection of single-cell colonies derived from 04981 or 25313 using indicated genotyping primers. Green forward: Puro-GT-R(LJL); Green reverse: PM20003-A-R-GT-R; Red forward: PM20003-A-L-GT-F; Red reverse: Puro-F. d, DNA agarose gel detection of single-cell colonies derived from 04981 or 25313 respectively, following removal of resistance cassettes, using indicated genotyping primers. Blue forward: PM20003-A-L-GT-F; Blue reverse: PM20003-A-L-GT-R3. *: mixed colonies containing cells with resistance cassettes not removed. Yellow boxes: single cell colonies used for following studies. e, Sanger sequencing validation of DMD K3584R mutant. Data represent 3 independent experiments. f, IB detection using indicated antibodies of GM04981 parental (Par) or K3584R mutant cells in the presence of indicated treatment. MG132 was used as proteasome inhibitor. g–i, Concentration measurement of H19 mimic or AGR-H19 per milligram of tissue at indicated time points post i.p. administration of H19 mimics (g), SubQ administration of H19 mimics (h), or SubQ administration of AGR-H19 (i) (10 mg/kg, single dose). Mean values±SD, n = 5 animals per time point per experimental condition. j, Left, representative images of α-bungarotoxin staining (top) or Duolink PLA (proximity ligation assay) using antibodies targeting LPR4 and Biotin respectively (bottom) of hiPS-SkMCs with indicated treatment. rAgrin, recombinant Agrin (Z+ form, aa. 1260–2045). Scale bars, 50 µm. Right: Percentage of cells with AchR clustering (blue bars) or Duolink PLA (red bars) of hiPS-SkMCs with indicated treatment. Mean values±SD, n = 6 independent experiments, two-way ANOVA, ***, p < 0.001. DNA agarose gel and immunoblots are representative of two independent experiments. Statistical source data and unprocessed immunoblots are provided as Source Data Extended Data Fig. 8.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | AGR-H19 and Nifenazone restore skeletal muscle and heart function. a, Immunohistological detection of Biotin-labeled mimics in quadriceps, heart, kidney, liver and lung tissues in the presence of indicated mimics. Scale bars, 100μm. Data represent independent replicates using 5 animals per group. b, Body weight measurement of male Dmd WT treated with scramble (Scr) or Dmd C3333Y mice with indicated treatments. Mean values±SD, n=5 mice per group, two-way ANOVA. c, d, Serum IGF1 (c) and IGF2 (d) level concentration of Dmd WT or Dmd C3333Y mice with the indicated treatments. Mean values±SD, n=5 mice per group, one-way ANOVA. e, RT-qPCR detection of Dmd in Dmd WT or Dmd C3333Y QUA (quadriceps) with indicated treatments. Mean values±SD, n=6 mice in each group, one-way ANOVA. f-i, RT-qPCR detection of indicated genes in Dmd C3333Y QUA with indicated treatments. Mean values±SD, n=5 (f, g, h) or 6 (i) mice in each group, one-way ANOVA. j, Serum CK concentration in Dmd WT treated with AGR-Scr or Dmd C3333Y mice with indicated treatments. Mean values±SD, n=5 mice per group, two-way ANOVA. k, Percentage of central nucleic of indicated muscle fiber of Dmd WT treated with AGR-Scr or Dmd C3333Y mice with indicated treatments. Mean values±SD, n=5 mice per group, two-way ANOVA. l, Body weight measurement of male Dmd WT mice treated with vehicle or Dmd C3333Y mice with indicated treatments from 4 weeks old to age of 16 weeks. Mean values±SD, n=5 mice per group, two-way ANOVA. m, Serum CK concentration in Dmd WT mice treated with vehicle or Dmd C3333Y mice with indicated treatments. Mean values±SD, n=5 mice per group, one-way ANOVA. No significance [n.s.], p > 0.05, *, p < 0.05, **, p < 0.01, ***, p < 0.001, and ****, p < 0.0001. Statistical source data are provided as Source Data Extended Data Fig. 9.
Extended Data Fig. 10 | AGR-H19 and Nifenazone improve the protein stability of dystrophin following exon-skipping. a, IP and IB detection using indicated antibodies of hiPS-SkMCs derived from healthy or DMD donor in the presence of biotinylated Scr or H19 mimics. b, Representative images of IF staining of hiPS-CMs derived from healthy donor treated with scramble and vehicle, or DMD patient (GM25313) treated with h44AON1 alone or in combination with H19 mimics or Nifenazone respectively. Scale bars, 100 μm. c, Statistical analysis of DMD (top) or Ub-DMD (Lys3584) (bottom) of hiPS-CMs derived from healthy donor (GM09503) treated with scramble and vehicle, or DMD patient (GM25313) treated with h44AON1 alone or in combination with H19 mimics or Nifenazone respectively. Mean values±SD. n = 5 independent experiments in each group, one-way ANOVA. d, IB detection using indicated antibodies of hiPS-SkMCs derived from healthy donor (H., GM09503) or DMD patient (25313) in the presence of h44AON1 and/or indicated treatment. e, IB detection using indicated antibodies of GM25313 parental (Par) or K3584R mutant cells in the presence of control (Ctrl.) or TRIM63 siRNA. hiPS-SkMCs derived from healthy donor (H., GM09503) were included as control. f, IB detection using indicated antibodies of GM25313 parental (Par) or K3584R mutant cells in the presence of control (Ctrl.) or TRIM63 siRNA. hiPS-SkMCs derived from healthy donor (H., GM09503) were included as control. MG132 was used as proteasome inhibitor. No significance [n.s.], p > 0.05, *, p < 0.05, **, p < 0.01, ***, p < 0.001. Immunoblots are representative of two independent experiments. Statistical source data and unprocessed immunoblots are provided as Source Data Extended Data Fig. 10.
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| ☒   | *Give P values as exact values whenever suitable.* |
| ☒   | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒   | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒   | Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated |

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

MARS Data Analysis Software v2.10 for CLARIOstar (BMG labtech) was used for calorimetric assay and optical density measurement. StepOne™ Software v2.3 (ThermoFisher) was used for data acquisition for Taqman genotyping PCR. CFX Manager™ Software v3.1 (BioRad) was used for data acquisition for quantitative PCR. AxioVision Software v4.8.2.0 (Carl Zeiss) was used for acquisition of microscopic images. Xcalibur v2.2 SP1.48 (Thermo Fisher Scientific) in cooperation with Q Exactive tune application version 2.2 SP1 Build 1646 (Thermo Fisher Scientific) was used for data acquisition for proteomics analyses. Exhaust treadmill running was used to evaluate the physiological activity of mice using the treadmill with X-PAK software v3.0 (Ugo Basile, 57631, Stoeiling). Surface Lead II ECG recordings were performed in consciousness, and freely mobile mice of 40 weeks old using implantable radio transmitters (EasyTEL S-ETA, 1.8 cc, 3.1g, EMKA Technologies). The ECG parameters were analyzed by the IOX (v1.0) Base software (EMKA Technologies). Excel version 2019 was used for data recording and analyze.

Data analysis

Image-pro plus 6.0 software (Media Cybernetics) was used for quantification of IHC staining density. Image J v1.5 was used for data analyses of western blot images. DNASTAR Lasergene12 Core Suite was used for Sanger sequencing data analysis. Proteome Discoverer version 1.3.0.339 (ThermoFisher Scientific) in cooperation with MASCOT search engine version 2.5.1.0 (Matrix Science) was used for proteomics data analyses. GraphPad Prism v8 and Excel were used for other data calculations and statistical analysis. The ECG parameters were analyzed by the IOX (v3.0) Base software (EMKA Technologies).

For manuscripts utilizing custom algorithms or software that are not central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Mass spectrometry data to identify DMD binding proteins and post-translational modifications have been deposited in ProteomeXchange with the primary accession code PXD020566 [ftp://ftp.pride.ebi.ac.uk/pride/archive/2020/08/PXD020566]. Source data are provided with this paper. All other data supporting the findings of this study are available from the corresponding authors on reasonable request.

Life sciences study design

Sample size

The sample sizes were determined by power analysis using StatMate version 2.0 (GraphPad Software). The experiment was set up to use 3-8 samples/repeats per experiment/group/condition to detect a 2-fold difference with power of 80% and at the significance level of 0.05 by a two-sided test for significant studies.

Data exclusions

No data were excluded from the data analysis.

Replication

Immunofluorescence staining experiments was independently repeated for 3-5 times or using 5-8 animals per experimental group. RNA Fluorescence in situ hybridization (FISH) coupled with immunofluorescence staining were performed 3 times independently. Immunoblotting detections were independently repeated 2 times. CLIP assays and EMSA assays were independently repeated 2 times. Transmission electron microscope data represents independent replicates using 5 animals per group. DNA agarose gel data represents 2 independent experiments.

Randomization

To obtain unbiased and reliable results, all the mice of the same age and the same gender were randomly grouped and housed in the same housing room.

Blinding

For the data quantification and animal treatments, investigators were blinded to group assignments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology and archaeology |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data |
| ☑   | Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

Antibodies

All the commercial antibodies were used following the manufacturer's instructions. Supplier name, catalog number and clone name are indicated here and in Supplementary Table 10.

DMD (Abcam ab15277, Polyclonal, IHC 1:200; IF 1:200; IB 1:1000, IP 1:100-1:2000; RIP 1:500)

Vinculin (ThermoFisher MAS-11690, clone VLN01, IF 1:200)

Ub-DMD (YenZym YZ6466, Polyclonal, IHC 1:100; IF 1: 100; IB 1: 500)
Validation

Antibody validations were performed by antibody suppliers. Ub-DMD (Lys3584) and Ub-TPSN antibody have been validated by peptide blocking assay.

DMD: https://www.abcam.com/dystrophin-antibody-ab15277.html
Vinculin: https://www.thermofisher.com/antibody/product/Vinculin-Antibody-clone-VLN01-Monoclonal/MA5-11690
GAPDH: https://datasheets.scbt.com/sc-32233.pdf
GST-Tag: https://www.cellsignaling.com/products/primary-antibodies/gst-26h1-mouse-mab/2624
His-Tag: https://www.thermofisher.com/antibody/product/Histone-H3-Antibody-Polyclonal/PAS-67671
FLAG-Tag: https://datasheets.scbt.com/sc-515946.pdf
beta-dystroglycan: https://www.scbt.com/p/betadystroglycan-antibody-47
SNTA1: https://www.thermoscientific.com/antibody/product/Synaptogmin-alpha-1-Antibody-Polyclonal/PA1-32071
SNTB1: https://www.origene.com/catalog/proteins/recombinant-proteins/tp324421/synaptogmin-sntb1-nm_021021-human-recombinant-protein
NOS1: https://www.scbt.com/p/nos1-antibody-a-11
alpha-sarcoglycan: https://www.scbt.com/p/alpha-sarcoglycan-antibody-d-7?requestFrom=search
beta-sarcoglycan: https://www.scbt.com/p/beta-sarcoglycan-antibody-f-6?requestFrom=search
gamma-sarcoglycan: https://www.scbt.com/p/gamma-sarcoglycan-antibody-expr17862-7-ab203113.html
delta-sarcoglycan: https://www.scbt.com/p/delta-sarcoglycan-antibody-b-5
alpha-dystrobrevin: https://www.scbt.com/p/alpha-dystrobrevin-iih6
alpha-dystroglycan: https://www.scbt.com/p/alpha-dystroglycan-antibody-4f7
beta-dystroglycan: https://www.scbt.com/p/beta-dystroglycan-antibody-4f7
delta-dystroglycan: https://www.scbt.com/p/delta-dystroglycan-antibody-4f7
MuRFl/TRIM63: https://www.thermoscientific.com/antibody/product/TRIM63-Antibody-Polyclonal/55456-1-AP
LRP4: https://www.abcam.com/lrp4-antibody-n2077-n-terminal-ab174637.html
anti-Biotin: https://www.abcam.com/biotin-antibody-ab53494.html
mouse-anti-myogenin-f5d: https://www.abcam.com/mouse-anti-myogenin-f5d/p/556358
Myogenin: https://www.bdbiosciences.com/us/applications/research/stem-cell-research/mesoderm-markers/human/purified-alpha-sarcolemmal-dystroglycan-1d2a-antibody-4f7
Nkx2.5: https://www.abcam.com/nkx2.5-antibody-ea53-ab9465.html
Sarcoglycan: https://www.scbt.com/p/alpha-sarcoglycan-antibody-d-7?requestFrom=search
beta-dystroglycan: https://www.scbt.com/p/beta-sarcoglycan-antibody-f-6?requestFrom=search
gamma-sarcoglycan: https://www.scbt.com/p/gamma-sarcoglycan-antibody-expr17862-7-ab203113.html
delta-sarcoglycan: https://www.scbt.com/p/delta-sarcoglycan-antibody-b-5
alpha-dystrobrevin: https://www.scbt.com/p/alpha-dystrobrevin-iih6
alpha-dystroglycan: https://www.scbt.com/p/alpha-dystroglycan-antibody-4f7
beta-dystroglycan: https://www.scbt.com/p/beta-dystroglycan-antibody-4f7
delta-dystroglycan: https://www.scbt.com/p/delta-dystroglycan-antibody-4f7
MuRFl/TRIM63: https://www.thermoscientific.com/antibody/product/TRIM63-Antibody-Polyclonal/55456-1-AP
LRP4: https://www.abcam.com/lrp4-antibody-n2077-n-terminal-ab174637.html
anti-Biotin: https://www.abcam.com/biotin-antibody-ab53494.html
K48-PolyUb: https://www.cellsignaling.com/products/primary-antibodies/k48-linkage-specific-polyubiquitin-antibody-4289
Ub-TPSN (Lys213) (YenZym, Polyclonal, IB 1:1000)
**Eukaryotic cell lines**

Policy information about cell lines

Cell line source(s)

C2C12 cells were purchased from American Type Culture Collection (ATCC). Skin fibroblast of healthy donor (GM05903) and human BMD patient’s donor fibroblasts cells (GM04569, GM05089, GM02298 and GM04981) and iP cells were obtained from Coriell Institute for Medical Research. 293FT cells were obtained from ThermoFisher (Cat # R70007).

Authentication

The cell lines were authenticated by short tandem repeats (STR) profiling performed by MDACC Characterized Cell Line Core Facility. The authenticity of iP lines and their derivatives (iPS-hepatocytes) were further confirmed by monitoring marker expression and conducting functional assays. 293 FT cells were authenticated by the vendor.

Mycoplasma contamination

All of the cell lines were free of mycoplasma contamination tested by MDACC Characterized Cell Line Core Facility using MycoAlert kit.

Commonly misidentified lines

No commonly misidentified cell line was used.

**Animals and other organisms**

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Both male and female Dmd WT and Dmd C3333Y mice were included in the experimental settings, male Mdx (C57BL/10ScSn-Dmdmdx/J mice and wild type C57BL/10J mice were included in the experimental settings. The age of animals we used in each experiment was indicated in the "Method". All animals were housed with a 12 h light/12 h dark cycle in the animal facility with free access to water and food. All of the mice were housed at temperatures of 65-75°F (~18-23°C) with 40-60% humidity.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field-collected samples.

Ethics oversight

All animal-based research is conducted according to the guidelines and requirements set forth by the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, the U.S. Department of Health and Human Services Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act of 1966 as amended by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas M.D. Anderson Cancer Center (MDACC). The study is compliant with all relevant ethical regulations regarding animal research.

Note that full information on the approval of the study protocol must also be provided in the manuscript.