SUPPLEMENTARY METHODS

Drp1-FGF21 axis drives FAPs-mediated fibro-adipogenic degeneration in Duchenne muscular dystrophy

Silvia Rosanna Casati, Silvia Zecchini, Christel Gentil, Oriola Gjana, Laura Lociuro, Michele Caccia, Zeno Lavagnino, Tilo Schorn, Diego Bottan, Pasquale Ogno, Harcel Djaya Mbissam, Emanuele Mocciaro, Dario Parazzoli, Davide Cervia, Emilio Clementi, France Pietri-Rouxel, Cristiana Perrotta, Matteo Giovarelli, Sestina Falcone and Clara De Palma.

Mice treatments

For mdivi-1 treatment, 12-week-old *mdx*-PhAM mice were treated by daily intraperitoneal (IP) injection of mdivi-1 (Merck, Darmstadt, Germany) at a concentration of 12.5 mg/kg per day for 8 weeks (1). The mice were weighed weekly to adjust the dose of the drug according to their weight. Mdivi-1 was dissolved in 5:5:90 dimethyl sulfoxide (DMSO)/cremophor/PBS vehicle, and fresh aliquots of the drug were prepared daily to avoid the freeze/thaw step. *Mdx*-PhAM male mice injected with 5:5:90 DMSO/cremophor/PBS vehicle represented the control group of the study. At the end of the treatment, the mice were subjected to a treadmill exhaustion test, and then they were sacrificed to collect their muscles for further experiments.

For AAV-shDrp1 treatment, the right tibialis anterior (TA) of 12-week-old *mdx*-PhAM mice was intramuscularly (IM) injected with a recombinant adeno-associated viral vector serotype 1 (AAV1), which has skeletal muscle tropism and remains episomal in the host cells(2), coding for a specific and already validated shRNA targeting the product of the Dnm1l gene (coding for Drp1 protein)(3) (4). The vector was purchased from Vector Biolabs (Burlingame, CA, USA) and it specifically contains a U6 promoter, the coding sequence for the shRNA to silence mouse Dnm1l 5'-CCG-GCG-GTG-GTG-CTA-GGA-TTT-GTT-ACT-CGA-GTA-ACA-AAT-CCT-AGC-ACC-ACC-GTT-TTT-3' and a sequence coding for mCherry fluorescent protein as a reporter gene. It was injected at a dose of 2.5 x 10^{11} gc (genome copies) in 25 µL of PBS per TA. Controls TA were injected with 25 µl of PBS. After 11 weeks from the injection, in *situ* TA muscle weakness and fragility tests were performed, and then the animals were sacrificed to collect TA muscles.

Muscle weakness and fragility

Muscle weakness (reduced specific maximal force) and fragility (susceptibility to contraction-induced injury) were evaluated by measuring the in *situ* TA muscle contraction in response to nerve stimulation. Mice were kept at a body temperature of 37 °C using radiant heating. The knee and foot were secured with pins and clamps, while the distal tendon of the target muscle was attached to a lever arm of a servomotor system (305B, Dual-Mode Lever; Aurora Scientific) using a silk ligature. The sciatic nerve was crushed proximally and stimulated distally with a bipolar silver electrode delivering supramaximal square-wave pulses (0.1 ms, 10 V). Maximal isometric force was recorded during tetanic contractions (125 Hz, 500 ms), with the muscle length set at L0, the length producing peak tension. Specific maximal force was calculated by normalizing absolute maximal force to muscle mass (5, 6).

To assess susceptibility to contraction-induced injury, force decline following lengthening contractions was measured. Stimulation of the sciatic nerve lasted 700 ms at 125 Hz. During the first 500 ms, a maximal isometric contraction of the TA muscle was elicited, followed by muscle stretching of 10% L0 over the final 200 ms at 5.5 mm/s (0.85 fiber lengths/s). A total of nine

lengthening contractions were applied, each separated by a 60-second rest interval, all performed at the initial muscle length L0. Force data were acquired using the PowerLab 8/36 system (AD Instruments), and the maximal force of each contraction was expressed relative to the initial maximal force (% of baseline) (5, 6).

Treadmill test

In the treadmill exhaustion test, animals were trained to run on the standard treadmill Exer 3/6 Treadmill (Columbus Instruments, Columbus, OH, USA), according to TREAT-NMD SOP DMD_M.2.1.003 (https://www.treat-nmd.org/wp-content/uploads/2023/07/MDX-DMD_M.2.1.003.pdf).

The test consisted of horizontal running for 5 min at 8 cm/sec, then the speed was increased by 2 cm/sec each minute, until the mice's exhaustion. Exhaustion was defined as the inability of the animal to return to running within 10 sec after direct contact with an electric stimulus grid. Running time and distance were provided by the software, while distance was calculated from time and speed. The recovery score of the parameters was calculated with the following formula: Recovery score = ([treated] − [untreated]) / ([normal] − [untreated]) × 100 according to the TREAT-NMD SOP (https://www.treat-nmd.org/wp-content/uploads/2023/07/*MDX*-DMD_M.1.1_001-21.pdf). Treated referred to mdivi-1-injected *mdx*-PhAM mice; untreated referred to the VEH-injected *mdx*-PhAM mice; normal referred to WT-PhAM animals. Mice were sacrificed at least 24 h after the exhaustion treadmill test.

Cell cultures and treatments

Muscle satellite cells and fibro-adipogenic precursors (FAPs) were prepared from 3-month-old *mdx*-PhAM mice following a standardized, automated tissue dissociation protocol with a gentleMACS™ Octo Dissociator with Heaters (Miltenyi Biotec, Bergisch Gladbach, Germany) and magnetic depletion as previously reported (7)

Satellite cells were plated on on Matrigel-coated culture dishes (Corning® Matrigel® Growth Factor Reduced Basement Membrane Matrix, Merck, Darmstadt, Germany) in growth medium (DMEM (EuroClone, Pero, Milan) supplemented with 20% fetal bovine serum (EuroClone, Pero, Milan), 3% chick embryo extract (USBiological life siences, Salem, MA, USA), 10 ng/mL basic fibroblast growth factor (PeproTech, London, UK), 1% L-Glutamine (EuroClone, Pero, Milan), and 1% penicillin-streptomycin (EuroClone, Pero, Milan)). Satellite cells were then treated with different concentrations of recombinant FGF21 (rFGF21) (PeproTech, London, UK) on Matrigel-coated Ibidi µ-Slide 8 Well (Ibidi GMBH, Gräfelfing, Germany). Two different experimental conditions were set: for the low-density condition, cells were seeded at a density of 10’000 cells/well in growth medium and treated for 48 h with either rFGF21 at final concentrations of 1 ng/mL, 10 ng/mL, 100 ng/mL, or with vehicle control (DMSO). Subsequently, the medium was replaced with differentiation medium (DMEM (EuroClone, Pero, Milan), 1% L-Glutamine

(EuroClone, Pero, Milan), and 1% penicillin-streptomycin (EuroClone, Pero, Milan), and 2% horse serum (EuroClone, Pero, Milan)) supplemented with rFGF21 or DMSO for another 48 h. For high-density conditions, cells were seeded at a density of 30'000 cells/well and directly supplemented with differentiation medium with either rFGF21 (1, 10, or 100 ng/mL) or DMSO for 48 h.

At the end of the treatments, the effects of rFGF21 on *mdx* satellite cells differentiation were evaluated by morphometric analysis. Myotubes were immunostained for skeletal muscle myosin and DAPI (ThermoFisher Scientific, Waltham, USA) to calculate the fusion index that was expressed as the ratio of the number of nuclei within the myotubes over the total number of nuclei.

FAPs were obtained from 3-month-old *mdx*-PhAM mice following the same dissociation process described for satellite cells. Following tissue digestion and filtration with a 30-µm cell strainer, FAP purification was performed using the satellite cells isolation protocol with some modifications. Specifically, cells were first subjected to magnetic depletion of lineage-positive populations, including $CD45^+$ hematopoietic cells, $CD31^+$ endothelial cells, and integrin $\alpha7^+$ myogenic cells (Miltenyi Biotec, Bergisch Gladbach, Germany). The lineage-negative fraction was subsequently enriched for Sca-1^+ cells by magnetic positive selection (Miltenyi Biotec, Bergisch Gladbach, Germany), yielding a purified FAP population. Cells were then plated for expansion in FAPs growth medium: DMEM (EuroClone, Pero, Milan) supplemented with 20% fetal bovine serum (EuroClone, Pero, Milan), 1% penicillin-streptomycin (EuroClone, Pero, Milan), 1% L-Glutamine (EuroClone, Pero, Milan), 10 mM Hepes, and 1 mM sodium pyruvate. The effects of rFGF21 on *mdx* FAPs adipogenic differentiation were evaluated through Oil Red O staining and RT-PCR assay. Briefly, cells were seeded on Ibidi µ-Slide 8 Well (Ibidi GMBH, Gräfelfing, Germany) (120'000 cells/well) for Oil Red O staining or on 12-well plates (216'000 cells/well) for RT-PCR assay in FAPs growth medium with either 1 ng/mL rFGF21 (PeproTech, London, UK) or DMSO. Cells were stopped after 72 h of treatment.

Nucleic acid extractions, RT-qPCR

Total RNA was isolated from frozen muscle samples or *mdx* FAPs using PureZOL reagent (Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions. Total RNA (500 ng) was retrotranscribed using the iScript gDNA Clear Reverse Transcription Supermix (Bio-Rad, Hercules, CA, USA). RT-qPCR was performed using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and the Real-Time QuantStudio 5 - 96-well 0.2 mL block instrument (ThermoFisher Scientific, Waltham, USA). All reactions were run as duplicates, and the fold changes were determined relative to the 36B4 housekeeping transcripts using the $2^{-\Delta\Delta CT}$ formula. In Table 1, the list of primers used is reported (Eurofin genomics, Ebersberg, Germany; Bio-Rad, Hercules, CA, USA).

Viral DNA was extracted from frozen muscle samples with Puregene Core Kit A (Qiagen, Hilden, Germany) following the manufacturer's instructions. Around 20 sections (thickens = 12 μm) of frozen TA have been used for each sample. The extracted DNA was quantified by Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, USA). The AAV viral genome was determined on 80 ng of gDNA with Master Mix TaqMan Fast Advanced (Applied Biosystems, Waltham, USA) and Real-Time QuantStudio 3 - 96-well 0.1 mL block instrument (ThermoFisher Scientific, Waltham, USA). Primers for Cytomegalovirus (CMV) promoter (FW: 5'-CATCAATGGGCGTGGATAGC-3' and Rev: 5'-GGAGTTGTTACGACATTTTGGAAA-3') and the TaqMan probe labeled with FAM (5'-ATTTCCAAGTCTCCACCC-3') were selected for the specific amplification of the vector genome sequence. Primers for titin gene (FW: 5'-TTCAGTCATGCTGCTAGCGC-3' and Rev: 5'-AAAACGAGCAGTGACGTGAGC-3') and TaqMan probe labeled with VIC (5'- TGCACGGAAGCGTCTCGTCTCAGTC-3') were selected for the normalization. The reaction mixes for each amplification reaction included 4 μL of diluted DNA (20 ng/uL) following the manufacturer's instructions. All reactions were run as duplicates, and the results were calculated using the $2^{-\Delta Ct}$ and the $2^{-\Delta\Delta Ct}$ methods in order to allow the normalization of each sample to titin gene.

Protein and mitochondria isolation and Western Blotting

Muscle samples were homogenized on ice with Ultra-Turrax (Ika-lab, Staufen, Germany) in 300-500 μL of lysis buffer (50 mM Tris-HCl pH 7.4 – 150 mM NaCl – 10 mM MgCl2 – 1 mM EDTA – 1% Triton X-100 – 0.5 mM DTT – 10% glycerol –2% SDS) supplemented with a cocktail of protease and phosphatase inhibitors (cOmplete and PhosSTOP, Roche Applied Science Mannheim, Germany). The samples were then incubated on ice for 30 min, sonicated for 10 sec, and centrifuged at 10,000 g for 10 min at 4°C. The supernatants were quantified using the DC Protein Assay (Bio-Rad, Hercules, CA, USA). 50 μg of total protein mixed with Laemmli buffer 4X were heated at 70°C for 5 min and loaded on either 4–20%, 10%, or 7.5% polyacrylamide precast gels (Criterion TGX Stain-free precast gels; Bio-Rad, Hercules, CA, USA) and transferred onto a nitrocellulose membrane using a Trans-Blot Turbo SystemTM and Transfer packTM (Bio-Rad, Hercules, CA, USA). For FGF21, 80 μg of total proteins were loaded and transferred onto a polyvinylidene difluoride (PVDF) membrane. The membranes were probed using the primary antibodies listed in Table 2. The bands were detected using horseradish-peroxidase-conjugated secondary antibodies and the Westar Antares or Westar Supernova ECL Substrates (Cyanagen, Bologna, Italy) with ChemiDocMP Imaging System (Bio-Rad, Hercules, CA, USA). Results were analyzed using the Image Lab software (Bio-Rad, Hercules, CA, USA). Uncropped original Western blots are provided as supplementary figures (Supplementary uncropped WB).

Muscles were dissected, cleaned of visible fat and connective tissue, minced with scissors in 1mL of Mito-buffer (100 mM KCl, 50 mM TRIS-HCl (pH 7.4), 5 mM MgCl2, 1.8 mM ATP, 1 mM

ethylenediaminetetraacetic acid (EDTA)) supplemented with Collagenase type V 0.1% (Merck, Darmstadt, Germany) in a 2 mL tube and then digested for 10 min at 37°C under strong agitation. After centrifugation at 380 x g for 5 min, the pellet was washed twice, suspended in 700 µl of Mito-buffer supplemented with a cocktail of protease and phosphatase inhibitors (cOmplete and PhosSTOP; Roche Applied Science Mannheim, Germany), and homogenized with Ultra-Turrax T10 (Ika-lab, Staufen, Germany) for 15 sec at maximum speed. Homogenized muscles were centrifuged for 5 min at 1,000 g at 4° C to discard nuclei, myofibrillar components, and fragments. The supernatant with mitochondria was centrifuged for 10 min at 10,000 g at 4°C: the pellet contained mitochondria (mitochondrial fraction), and the supernatant contained cytosolic proteins (cytosolic fraction). The mitochondria pellets were resuspended in 0.5 mL of Mito Preservation Medium (MiR06 supplemented with 20 mM Histidine, 20 µM Vitamin E succinate, 3mM Glutathion; 1µM Leupetine, 2 mM Glutamate, 2mM Malate, 2 mM Mg-ATP) for high-resolution respirometry analysis. For protein analysis, mitochondrial pellets were solubilized in 50 ul of RIPA lysis buffer (150 mM NaCl – 5 mM EDTA pH 8 – Tris-HCl pH 8 – 1% NP-40 – 0.5% sodium deoxycholate – 0.1% SDS in dH2O) supplemented with a cocktail of protease and phosphatase inhibitors (cOmplete and PhosSTOP; Roche Applied Science, Mannheim, Germany).

High-resolution respirometry (HRR)

Oxygen consumption rates were measured by high-resolution respirometry (HRR) using 200 µg of isolated mitochondria from TA muscle resuspended in mitochondrial respiration medium MiR06 (0.5 mM EGTA - 3 mM MgCl2 - 60 mM Klactobionate - 20 mM taurine - 10 mM KH2PO4 20 mM Hepes - 110 mM sucrose and 1 g/l bovine serum albumin fatty acid-free, - 280 U/mL catalase (pH 7.1)). The respiration rates of mitochondria were measured in the 2mL O2K oxygraph chambers (Oroboros Instruments, Innsbruck, Austria) at 37 °C. Titrations of substrates, uncouplers, and inhibitors were added in series. We added glutamate (10 mM), malate (2 mM), and ADP (2.5 mM) to obtain complex I (CI) activity. The addition of cytochrome C (10 µM) was performed to test the integrity of the outer mitochondrial membrane. The addition of complex II substrate, succinate (10 mM), allowed the measurement of the maximal OXPHOS capacity (CI+II). Then, CI was inhibited by injecting rotenone 0.5 µM (CII). To measure leak state (L), we added oligomycin (10 nM), an ATP synthase inhibitor. Antimycin A (2.5 µM) was added to inhibit CIII, thus obtaining residual oxygen consumption. Complex IV (CIV) activity was stimulated by using N,N,N0,N0-Tetramethyl-p-phenyl-enediamine dihydrochloride (TMPD) (0.5 µM) and ascorbate (2 mM). Oxygen fluxes were corrected by subtracting residual oxygen consumption from each steady state. The DatLab7 software (Instruments Oroboros, Innsbruck, Austria) was used for data acquisition and analysis.

Proximity Ligation Assays (PLA)

Single fibers were obtained from gastrocnemius (GS) muscles, previously fixed (4% paraformaldehyde, PBS) for 2 h at room temperature, and then permeabilized (1% Triton X-100, PBS) for 2 h. Permeabilized fibers were blocked with the Duolink Blocking Solution (Merck, Darmstadt, Germany) for 1 h at 37°C. Primary antibodies anti-Drp1 (Mouse, BD Bioscience, San Jose, CA, USA) and anti-Fis1 (Rabbit, Enzo Life Science, New York, USA) were diluted 1:100 in the Duolink Antibody Diluent prepared in Duolink Blocking Solution (Merck, Darmstadt, Germany) and were incubated O/N at 4°C. After the washing steps, the fibers were incubated with PLA probe solution (Duolink plus and minus PLA probes diluted 1:5 in the Duolink Antibody Diluent) for 1 hour at 37°C. Fibers were then incubated with the ligation solution (Duolink ligase diluted 1:40 in Duolink Ligation buffer 1X) for 37 min at 37°C and then the amplification solution (Duolink polymerase diluted 1:80 in Duolink Amplification buffer 1X) for 100 min at 37°C. Next, fibers were washed in Milli-Q water and mounted on glass slides with Fluoroshield mounting medium (Merck, Darmstadt, Germany). Several Z-stack images of each fiber were acquired with Leica TCS SP8 AOBS microscope system with a 40X/1.30 oil objective (Leica Microsystems, Wetzlar, Germany). The analysis was performed on the Z-projections of each image, setting an appropriate threshold that was used for all the images. ImageJ software was used for this analysis, and the normalization was performed on the area of the fiber.

Histology and immunofluorescence

Dissected muscles (TA and DP) were immediately frozen to allow the preparation of 10 μm thick sections by Leica CM1860 UV cryostat (Leica Biosystems, Milan, Italy) for both histological and immunofluorescence analysis

Hematoxylin and Eosin (H&E) staining (Bio Optica, Milan, Italy) was performed as previously described (8). Sirius Red staining was used to quantify fibrosis: sections were fixed in 4% formaldehyde (Merck, Darmstadt, Germany) for 15 min at room temperature, washed in distilled water, and ethanol 100% (5 min). Once dried, sections were stained with 0.3% Sirius Direct Red 80 picric acid solution (Merck, Darmstadt, Germany) for 1 hour at room temperature, rinsed in distilled water, dipped twice in 0.5% acetic acid (5 min), 100% EtOH (5 min), and ultimately cleared in Xylene (10 min), and mounted using Eukitt mounting medium (Merck, Darmstadt, Germany). Images were acquired with NanoZoomer®-SQ (Hamamatsu Photonics K.K., Japan) and NDP.view2 software.

For immunofluorescence, sections or satellite cells were fixed with 4% paraformaldehyde for 15 min at room temperature, blocked for 1 hour with 5% goat serum, 0.1% Triton X-100, PBS, and then incubated with primary antibodies diluted in blocking solution O/N at 4°C. For MYH3 staining, muscle sections were not fixed. The primary antibodies for laminin, CD45, MYH3, perilipin, and myosin (MF20) are listed in Table 2. After incubation with the appropriate

secondary antibodies conjugated to Alexa Fluor (Thermo Fisher Scientific, Waltham, USA), slides or Ibidi μ-Slide 8 Well were mounted with Fluoroshield mounting medium (Merck, Darmstadt, Germany). When necessary, before mounting, the nuclei were counterstained with DAPI (1:1000 for 10 min).

Oil Red O (Merck, Darmstadt, Germany) was used to visualize lipid accumulation. Cells were fixed in 4% paraformaldehyde for 15 min at room temperature, rinsed in 60% isopropanol, stained with Oil red O working solution for 45 min, and rinsed in water. The Oil Red O working solution was freshly prepared by diluting a stock solution (3 mg/mL Oil Red O dissolved in isopropanol) 1:3 with distilled water. The Oil Red O positively stained area was quantified using ImageJ software. Normalization was performed on the total number of nuclei.

Images were acquired using Leica TCS SP8 AOBS microscope using a 40X/1.30 oil immersion objective (Leica Microsystems, Wetzlar, Germany). ImageJ software was used to analyze images, setting an appropriate threshold for all the images. Laminin analysis for cross-sectional area has been performed with Aivia 15.0.0 software.

Mitochondria network analysis

Fiber bundles were obtained from soleus muscles previously fixed (4% paraformaldehyde, PBS) for 2 h at room temperature. Single fibers were then mounted on a glass slide using Fluoroshield mounting medium (Merck, Darmstadt, Germany) and a coverslip. Mitochondrial network images were acquired using Leica TCS SP8-DLS and Leica Stellaris 8 DLS, both equipped with HC PL APO CS2 63x/1.40 oil immersion objective and lightning correction (Leica Microsystems, Wetzlar, Germany). For each fiber, 12 Z-stacks were acquired at 0.27 μm.

For the analysis of mitochondrial network organization, the directionality plugin of ImageJ was used to measure mitochondrial dispersion (a measure of disorganization) on Z-projection. Furthermore, the images were processed using a home-made MATLAB code. After importing the data, the Z-projection of each image was used to identify the fiber area to be analyzed. The resulting images have been linearized along the fiber axis and considered as fluctuating fluorescent signals whose features have been investigated by means of autocorrelation functions. The autocorrelation function reveals that, statistically, the signal in space can be described as a superimposition of oscillatory patterns subject to decay. Horizontal asymptote was used to evaluate oscillation persistence: the slower the decay, the higher the value of the horizontal asymptote, and the greater the regularity of the mitochondrial network spatial organization.

For the mitochondrial photoconversion experiment, an Olympus Spinning Disk CSU mounted on an Olympus IX83 inverted microscope equipped with a 100x/1.35 NA silicon immersion objective (Advanced Light Microscopy facility, IFOM ETS - the AIRC Institute of Molecular Oncology) was used to photoconvert small regions of the mitochondrial network (ROI: 0.84 x

0.84 µm) in freshly dissected soleus fiber bundles. Photoconversion (minimum of eight ROIs per image) was performed using a 405 nm laser. During the experiment, fiber bundles were maintained at 37 °C and 5% CO2. A 2-minute time-lapse movie was recorded with 20s time-frame setting. We measured with ImageJ the spread of the red signal after the photoconversion as a measure of mitochondrial network interconnectivity. Low mitochondrial connectivity is associated with reduced red signal diffusion in nearby mitochondria.

1. Giovarelli M, Zecchini S, Martini E, Garre M, Barozzi S, Ripolone M, et al. Drp1 overexpression induces desmin disassembling and drives kinesin-1 activation promoting mitochondrial trafficking in skeletal muscle. Cell Death Differ. 2020.
2. Leduc-Gaudet JP, Reynaud O, Hussain SN, Gouspillou G. Parkin overexpression protects from ageing-related loss of muscle mass and strength. J Physiol. 2019;597(7):1975-91.
3. Dulac M, Leduc-Gaudet JP, Reynaud O, Ayoub MB, Guerin A, Finkelchtein M, et al. Drp1 knockdown induces severe muscle atrophy and remodelling, mitochondrial dysfunction, autophagy impairment and denervation. J Physiol. 2020;598(17):3691-710.
4. Dulac M, Leduc-Gaudet JP, Cefis M, Ayoub MB, Reynaud O, Shams A, et al. Regulation of muscle and mitochondrial health by the mitochondrial fission protein Drp1 in aged mice. J Physiol. 2021;599(17):4045-63.
5. Forand A, Moog S, Mougenot N, Lemaitre M, Sevoz-Couche C, Guesmia Z, et al. Long-Term Dystrophin Replacement Therapy in Duchenne Muscular Dystrophy Causes Cardiac Inflammation. JACC Basic to translational science. 2025;10(6):759-82.
6. Traore M, Noviello C, Vergnol A, Gentil C, Halliez M, Saillard L, et al. GDF5 as a rejuvenating treatment for age-related neuromuscular failure. Brain. 2024;147(11):3834-48.
7. Zecchini S, Giovarelli M, Perrotta C, Morisi F, Touvier T, Di Renzo I, et al. Autophagy controls neonatal myogenesis by regulating the GH-IGF1 system through a NFE2L2- and DDIT3-mediated mechanism. Autophagy. 2019;15(1):58-77.
8. Giovarelli M, Zecchini S, Catarinella G, Moscheni C, Sartori P, Barbieri C, et al. Givinostat as metabolic enhancer reverting mitochondrial biogenesis deficit in Duchenne Muscular Dystrophy. Pharmacol Res. 2021;170:105751.