Eur J Transl Myol 33 (3) 10501, 2023 doi: 10.4081/ejtm.2023.11501

# Autophagy increase in Merosin-Deficient Congenital Muscular Dystrophy type 1A

Mariangela Mastrapasqua (1), Roberta Rossi (2), Lucrezia De Cosmo (3), Annalisa Resta (4), Mariella Errede (1), Antonella Bizzoca (1), Stefania Zampatti (5), Nicoletta Resta (6), Emiliano Giardina (5), Maddalena Ruggieri (1), Daniela Virgintino (1), Tiziana Annese (1,7), Nicola Laforgia (8), Francesco Girolamo (1)

*(1) Department of Translational Biomedicine and Neuroscience (DiBraiN), University of Bari 'Aldo Moro', Bari, Italy; (2) Section of Pathology, Department of Precision and Regenerative Medicine and Jonian Area (DiMePRe-J), University of Bari "Aldo Moro", Bari, Italy; (3) Neonatology and Neonatal Intensive Care Unit, Ospedale SS. Annunziata, Taranto, Italy; (4) Neonatology and Neonatal Intensive Care Unit, Ospedale Miulli, Acquaviva delle Fonti, Italy; (5) Laboratory of Genomic Medicine – Santa Lucia Foundation – IRCCS, Roma, Italy; 6Medical Genetics Unit, Department of Precision and Regenerative Medicine and Ionian Area (DiMePRe-J), University of Bari "Aldo Moro", Bari, Italy; (7) Department of Medicine and Surgery, Libera Università del Mediterraneo (LUM) Giuseppe Degennaro University, Bari, Italy; (8) Neonatology and Neonatal Intensive Care Unit, Department of Interdisciplinary Medicine (DIM), University of Bari "Aldo Moro", Bari, Italy.*



***Abstract***

The autophagy process recycles dysfunctional cellular components and protein aggregates by sequestering them in autophagosomes directed to lysosomes for enzymatic degradation. A basal level of autophagy is essential for skeletal muscle maintenance. Increased autophagy occurs in several forms of muscular dystrophy and in the merosin-deficient congenital muscular dystrophy 1A mouse model (dy3k/dy3k) lacking the laminin-α2 chain. This pilot study aimed to compare autophagy marker expression and autophagosomes presence using light and electron microscopes and western blotting in diagnostic muscle biopsies from newborns affected by different congenital muscular myopathies and dystrophies. Morphological examination showed dystrophic muscle features, predominance of type 2A myofibers, accumulation of autophagosomes in the subsarcolemmal areas, increased number of autophagosomes overexpressing LC3b, Beclin-1 and ATG5, in the merosin-deficient newborn suggesting an increased autophagy. In Duchenne muscular dystrophy, nemaline myopathy, and spinal muscular atrophy the predominant accumulation of p62+ puncta rather suggests an autophagy impairment.

**Key Words**: autophagy; congenital muscular dystrophy; LC3; p62; Beclin-1.

*Eur J Transl Myol 33 (3) 10501, 2023 doi: 10.4081/ejtm.2023.11501*

Congenital myopathies and muscular dystrophies are hereditary myopathies featuring weakness, hypotonia, and characteristic features on muscle biopsy.[1] In Italy, congenital merosin-deficient muscular dystrophy (MDC1A; OMIM #607855) is a rare disease with a prevalence of 0.136 per 100,000 in the all-age population accounting for 24.11% of congenital muscular dystrophies (CMDs) [2,3] in which laminin-α2 (LAMA2), a large glycoprotein of the basal lamina, is reduced or absent.[4,5] Nemaline myopathies (NMs) are a heterogeneous group of CMDs caused by de novo dominant or recessive mutations in at least 12 genes being similarly rare muscle diseases with an overall prevalence of 0.2 per 100.000.[6] Another congenital disease presenting with profound muscle weakness derives from irreversible loss of motor-neurons of spinal cord and brain stem nuclei (spinal muscular atrophy type 0, SMA 0) being one of the diseases for primary differential diagnosis with CMDs.

Autophagy is a catabolic pathway in which altered intracellular components are sequestered in autophagolysosomes for degradation. In the LAMA2

***Table 1.*** *Clinical, laboratory and morphometric findings of patients.*

| Diagnosis | Age at biopsy | Sex | Onset clinical presentation | Summary of MRI findings | Creatine kinase at biopsy time (IU/L)[2] |
|---|---|---|---|---|---|
| MDC1A (congenital merosin-deficient muscular dystrophy) | 10 days | M | Significant postnatal hypotonia, weak feeding and crying | Normal signal intensities on T1--and T2-weighted images of brain GM and WM | 43765 |
| NEM10 (Nemaline myopathy 10) | 8 days | F | Low foetal movements, significant postnatal hypotonia, no spontaneous movements and no elicitable reflexes, weak feeding | Normal signal intensities on T1--and T2-weighted images of brain GM and WM | ND |
| SMA 0 (Spinal muscular atrophy 0) | 13 days | F | Respiratory distress at birth and significant postnatal hypotonia | Normal signal intensities on T1--and T2-weighted images of brain GM and WM | ND |
| DMD (Duchenne-type muscular dystrophy) | 5 years | M | Frequent falls, kyphoscoliosis, waddling gait, respiratory distress | ND | 792 |
| DMD (Duchenne-type muscular dystrophy) | 7 years | M | Weakness, short stature, difficult running, Gowers sign, | Neck flexors, biceps, triceps, quadriceps hypotrophy and fibro-fatty replacement | 2189 |
| IMNM (Immune-mediated necrotizing myopathy) | 81 | F | Limb proximal weakness, myalgia | Myofascial quadriceps, harmstring, and gluteal oedema. | 2416 |
| CTRL (control muscle tissue) | 24 years | M | Asymptomatic, occasional elevated serum creatine kinase | ND | 1543 |

deficient mouse (dy3k/dy3k) model of MDC1A, there is increased expression and function of autophagy molecules; systemic administration of 3-methyladenine, a PI3K inhibitor of autophagosome formation, improves the dystrophic phenotype, increasing lifespan and motility.[7] To determine whether an enhanced expression of autophagy-related molecules is a general, aspecific reaction of myofibers to different noxae or limited to specific muscle dystrophies, we analyzed by immunohistochemistry (IHC), immuno-electron microscopy (IEM) and western blotting (WB), the expression of LC3b, a late phase marker of autophagy and p62/SQSTM1, a shuttle receptor of LC3. Beclin-1, an early phase marker of phagophore formation and ATG5, an intermediate phase marker, were also morphologically evaluated. We compared the densities of IHC expression of LC3 and p62 in diagnostic muscle biopsies of newborns affected by MDC1A, NM10, SMA 0 with biopsies of Duchenne muscular dystrophy (DMD)-affected boys, and control muscle tissues.

## Materials and Methods

Single open biopsies of muscle tissue obtained for diagnostic purposes from 3 newborns, 2 children, and 2 adult patients were retrospectively reviewed. The patients had clinical, pathological, and genetic confirmation of a specific myopathy (Table 1). Three newborns are affected by MDC1A, NM10, and SMA 0, the other two by DMD. As positive control of autophagy marker accumulation, we used muscle tissues from an adult patient diagnosed with immune-mediated necrotizing myopathy (IMNM).[8] A normal control specimen was derived from an apparently healthy subject with mildly elevated serum levels of CK but without any histological abnormality, including the absence of myonecrosis, dystrophic features, and inflammatory infiltrates (Tables 1, 2 and 3). All procedures performed in this study were in accordance with the ethical standards of the 1964 Helsinki Declaration and later amendments. All patients or their relatives signed

***Table 2.*** *Myopathological, genetic, and morphometric findings of patients.*

| Diagnosis | Summary of myopathological findings | Molecular genetic testing | Mean area fraction of LC3b$^+$ puncta | Mean number of LC3b$^+$ puncta per myofiber | Mean LC3b$^+$ puncta diameter | % of myofibers with LC3b$^+$ puncta |
|---|---|---|---|---|---|---|
| MDC1A | necrotic myofibers, myophagia, fibrosis, atrophy, hypertrophy, predominance of type 2A fibers | 2901C>A p.(Cys967Ter); 5050G>T p.(Glu1684Ter) in the *LAMA2* gene | 8.6 | 21.07±11.81 | 0.48 | 38.14 |
| NEM10 | necrotic myofibers, fibrosis, severe atrophy, mild hypertrophy | homozygous c.1648C>T, p.(Leu550Phe) in the *LMOD3* gene | 3.07 | 3.6±2.07 | 0.42 | 8.1 |
| SMA 0 | myofiber atrophy, hypertrophy, subsarcolemmal basophilia | compound heterozygote :c.815A>G;p.Y272C and 1 *SMN1* copy (deletion) 1 copy *SMN2* | 5.05 | 1.54±092 | 0.32 | 1.52 |
| DMD | myofiber atrophy, central nuclei | r.(650_2168del) in the *dystrophin* gene | 10.21 | 7.67±5.86 | 0.51 | 8.13 |
| DMD | fibrosis, fatty infiltration, myofiber atrophy, hypertrophy, dark myofibers, predominance of type 1 fibers | r.(475_476del) in the *dystrophin* gene | 9.23 | 5.48±6.23 | 0.47 | 7.46 |
| IMNM | necrotic myofibers, ghost fibers, atrophy, hypertrophy, endo- and perimysial inflammatory infiltrates. | NA | 11.32 | 15.64±8.35 | 0.95 | 4.38 |
| CTRL | no pathologic findings | NA | 0.28 | 1.22±2.10 | 0.15 | 0.5 |

informed consent for diagnostic and research analyses and specimen inclusion in a muscle biobank.

### *Muscle Biopsy Analysis*

The muscle biopsy specimens were rapidly frozen in isopentane, cooled in liquid nitrogen, and kept at −80°C until use. Serial, transverse, unfixed sections underwent standard histochemical and IHC stainings, as previously detailed.[9,10] Primary antibodies diluted in blocking buffer were used to recognize Beclin-1 (1:10; Abcam, code ab51031; Cambridge, United Kingdom), ATG5 (1:100; Abcam, code ab78073), LC3b (1:300; Abcam, code ab48394), p62/SQSTM1 (1:50; Abcam, code ab56416), laminin-α2-β1-γ1 (1:200; Sigma-Aldrich, code L9393; Darmstadt, Germany), laminin-α2 (merosin; 1:20; Monosan, clone Mer3/22B2; Uden, The Netherlands), laminin-α1 (1:200; Novus Biologicals, code CL3183; Minneapolis, MN, USA) and laminin-α4 (Novus Biologicals, code NBP2-42393). Negative controls were prepared by omitting the primary antibodies, pre-adsorbing the primary antibodies with an excess of Tableantigen when available, and mismatching the secondary antibodies. Immunolabelled sections were

***Table 3.*** *Other morphometric findings of patients.*

| Diagnosis | Mean area fraction of $p62^+$ puncta | Mean number of $p62^+$ puncta per myofiber | Mean $p62^+$ puncta diameter | % of fibers with $p62^+$ puncta | % of necrotic myofibers | % of type 2A myofibers |
|---|---|---|---|---|---|---|
| MDC1A | 1.82 | 2.75±5.73 | 0.35 | 6.42 | 7.04 | 61.1 |
| NEM10 | 1.25 | 4.04±4.24 | 0.36 | 14.27 | 1.28 | 6.8 |
| SMA 0 | 1.32 | 3.33±3.21 | 0.34 | 13.07 | 0.14 | 7.5 |
| DMD | 2.64 | 6.5±1.97 | 0.58 | 19.98 | 1.15 | 29.7 |
| DMD | 2.72 | 5.07±1.88 | 0.61 | 18.92 | 0.96 | 35.8 |
| IMNM | 5.21 | 12.85±7.58 | 0.75 | 2.45 | 1.97 | 39.2 |
| CTRL | 0.58 | 0.6±0.89 | 0.14 | 0.91 | 0.0 | 22.8 |

photographed under a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems, Mannheim, Germany) using a sequential scan procedure. Confocal images were taken with 20x, 40x, 63x oil lenses applying digital zoom. Z-stacks of serial optical planes (projection images) and single optical planes were analysed by ImageJ software (NIH, Bethesda, MD, USA). Quantitative evaluations were performed by F.G., blinded for patient characteristics and diagnosis in all biopsies, by means of computer-aided morphometry applied to microscopic images. Positive puncta were counted on 30 myofibers at least using the default 'analyze particles' plugin in ImageJ software. Puncta with an area of 0.1–1.767 $\mu m^2$ were quantitated for LC3b and p62. Results were expressed as average values ± SD. No statistical analysis was performed.

### *IEM Analysis*

Frozen muscle specimens from the MDC1A and NM10 were fixed in 2.5% glutaraldehyde and processed as previously detailed.[11] Ultra-thin sections were observed using a transmission electron microscopy Morgagni 268 (FEI Company, Hillsboro, OR, USA). Immunoelectron microscopy was performed as previously detailed.[12] Briefly, ultrathin sections were mounted on formwar-coated gold grids and incubated overnight with the LC3b antibody (Abcam) diluted 1:300 in blocking buffer (TBS 0.1 M, pH 7.4 + BSA1%) at 4 °C. After rinsing with TBS (0.1 M, pH 7.4), the grids were incubated at a dilution of 1:20 of 20-nm gold-conjugated anti rabbit IgG (Sigma) in TBS for 1 hr at RT. After several rinses in TBS, the grids were lightly stained with uranyl acetate and lead citrate. The grids were observed under a Morgagni 268 electron microscope (FEI).

### *WB Analysis*

Frozen muscle specimens (20 mg) from the MDC1A, NM10, anti-$HMGCR^+$ IMNM, DMD, and control biopsy were transferred into precooled tubes and immediately solubilized by using an Ultra Turrax T8 durable homogenizer (IKA, Wilmington, North Carolina) in at least 10 volumes of extraction buffer (radio-immuno-precipitation assay buffer; Thermo Fisher Scientific, Waltham, Massachusetts) added with a cocktail of protease inhibitors (Roche Diagnostics, Mannheim Germany). The immunoblotting method was previously detailed.[9]

The membranes containing the blotted proteins were incubated with primary antibodies LC3b (1:750; Abcam), p62 (1:1000; Abcam), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:3,000; Sigma, St Louis, Missouri) overnight at 4°C. After having been washed with 0.1% Tween 20 PBS, the membranes were incubated with near-infrared fluorescent secondary antibodies IRDye 800CW anti-rabbit (LI-COR, Lincoln, Nebraska) or IRDye 800CW anti-mouse (1:7,000; LI-COR) secondary antibodies for 1 h at room temperature. For immunoblotting analysis, the LI-COR Odyssey infrared imaging system was used.

## Results

The newborns were admitted to the neonatal intensive care unit of Bari University Hospital for severe hypotonia, respiratory distress at birth and weak feeding (Table 1). On admission, complete blood count, serum electrolytes, bilirubin, lactic acid, and ammonium levels were normal. Muscle enzymes were increased, especially CK levels (Table 1). Needle electromyography showed abnormal findings, whereas brain MRI was normal (Table 2). Muscle biopsy was performed, demonstrating the presence of necrotic myofibers and other specific findings (Table 3). The impact of muscle biopsy on the diagnostic workflow was evident in the MDC1A patient, whose muscle tissue showed a complete lack of LAMA2 immunohistochemical staining around myofibers (Suppl. Fig. 1A, C).

Compared to the control muscle (Suppl. Fig. 1B), a compensatory hyper-expression of laminin-α1 and -α4 (Suppl. Fig. 1D, E) was observed, together with hyper-expression of the other laminin subunits, detected using a pan-laminin antibody (Suppl. Fig. 1A). In addition, in the MDC1A muscle biopsy, immunofluorescence labelling showed that myofibers accumulated compartmentalized puncta positive for Beclin-1 (Fig. 1A), ATG5 (Fig. 1B), LC3b (Fig. 1C, F), and p62 (the shuttle receptor between LC3 and ubiquitinated proteins, Fig. 1E, L). Indeed, the comparison of different morphological parameters related to immunolabelling for

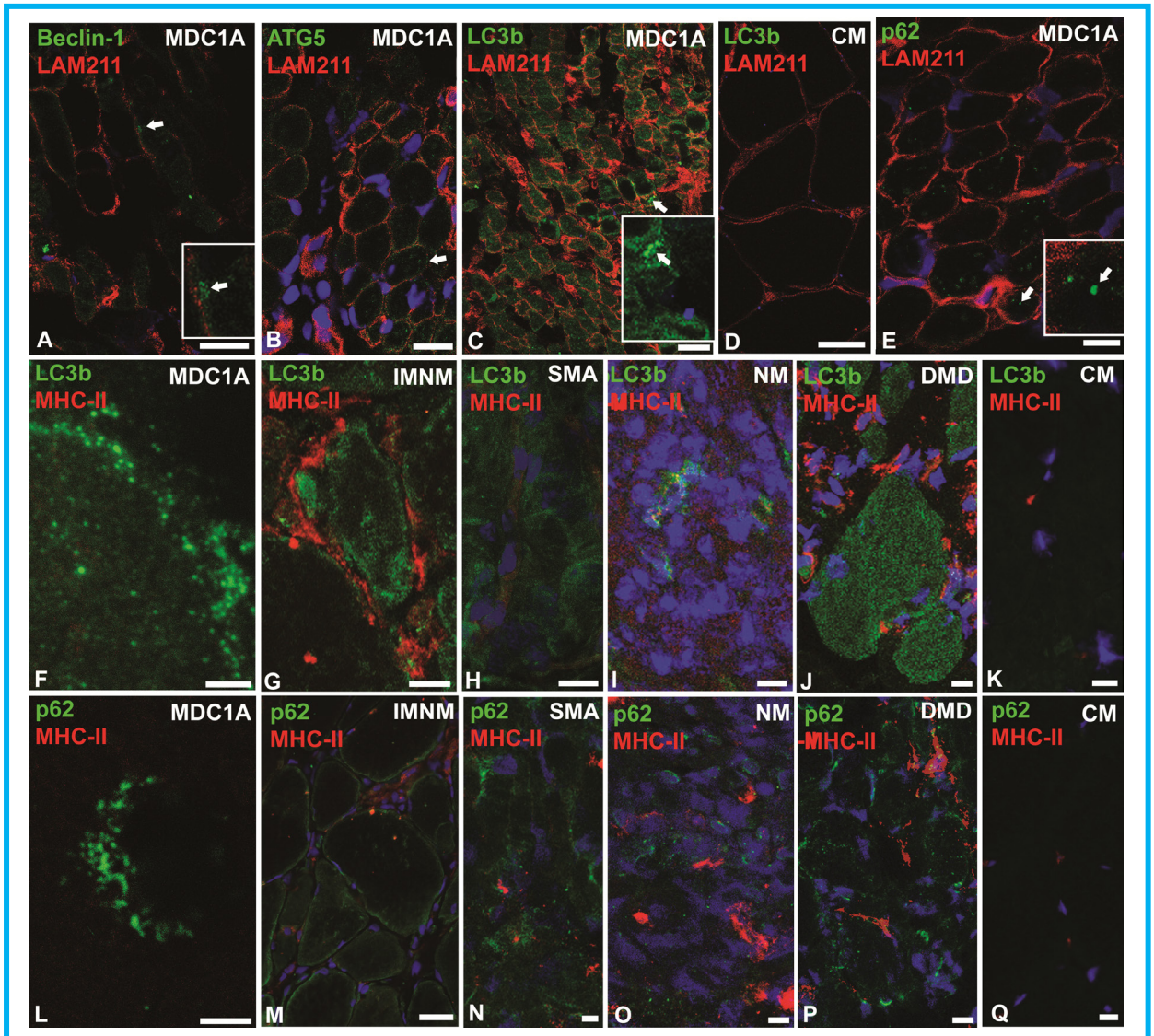



***Fig 1.*** *Comparison of autophagy markers immunolabelling among different neuromyopathies. (A-C, E, F, L) Sections of MDC1A muscle biopsy double immunolabeled with anti-pan-laminin (red channel in A-C, F, M) and 4 different autophagy markers (green channel) such as anti-Beclin-1 (A; Abcam, code ab51031), an early phase phagophore formation marker, anti-ATG5 (B; Abcam, code ab78073), intermediate phase marker, anti-LC3b (C, F; Abcam, code ab48394), late phase marker, and anti-p62/SQSTM1 (E, L; Abcam, code ab56416), a shuttle receptor of LC3 from ubiquitin pathway. These different autophagy markers localize as green stained puncta in sarcoplasms of several myofibers (arrows). (C, F-J) LC3b+ stained puncta are more frequently seen in MDC1A and IMNM than DMD, SMA, and NM. (E, L-P) p62+ stained puncta are more frequently seen in DMD and IMNM than the other neuromyopathies. (D, K, Q) No LC3 and p62 immunostainings are normally detectable in sarcoplasmic adult healthy control muscle (CM). (F-Q) Both LC3b and p62 appeared preferentially localizing in proximity to MHC-II+ endomysial cells in IMNM (G, M), but separated in the other neuromyopathies. Scale bars: A-E: 50 µm; insets horizontal side in A (27 µm), C (47 µm), E (67 µm); F, L: 2 µm; G-K, M-Q: 10 µm.*

two significant autophagy markers, LC3b and p62, among the different neuromyopathies revealed the highest density of compartmentalized puncta and % of myofibers accumulating LC3b in MDC1A compared to the other infantile neuromyopathies, whereas p62 appeared less expressed than LC3b in MDC1A (Fig. 1; Table 1). Adult control muscle was unstained with these autophagy markers (Fig. 1D, K, Q). A notable invasion of MHC-II$^{+}$ inflammatory cells was noted in the analyzed neuromyopathies with no preferential association to autophagy puncta (Fig. 1F, H-J, L, N-P), an interesting feature instead noted in IMNM myofibers (Fig. 1G, M).

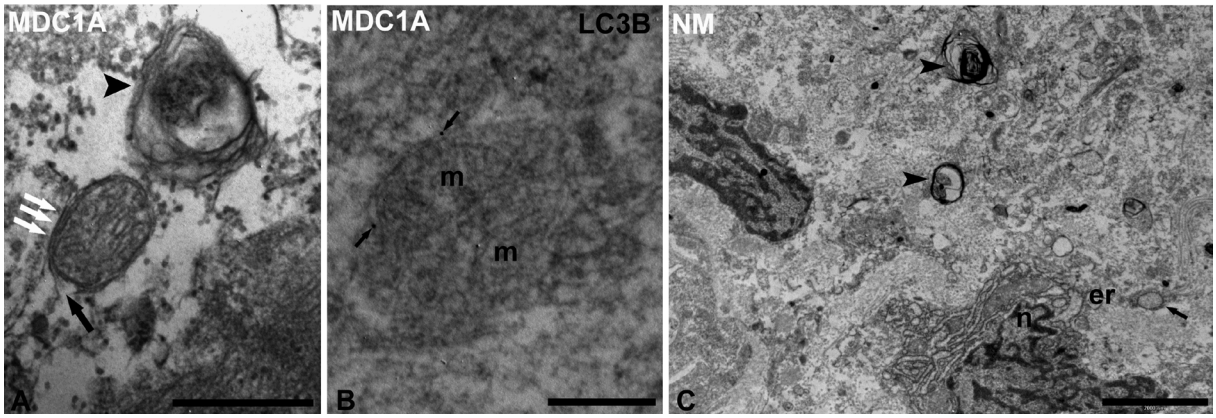



***Fig 2.*** *Transmission electron microscopy (TEM) images of autophagy. (A) The TEM image obtained from frozen sample of the MDC1A-affected muscle biopsy shows an autophagosome (arrowhead) appearing as a multilayered vesicle containing electron-dense material. In an earlier step, a three-layered membrane phagophore (white arrows) appears as an open bag (black arrow) enclosing a mitochondrion. (B) Immunogold electron microscopy image from the same MDC1A patient shows LC3B immune-conjugated 20 nm nanoparticles (arrows) localized on a membrane enclosing two mitochondria (m). (C) Multilayered membrane autophagosomes (arrowheads) are also localized in the perinuclear region (n) of myofibers from NM-affected newborn, where an early endosome (arrow) and dilated cisterns of endoplasmic reticulum (er) appeared in close proximity to the nuclear membrane. Scale bars: A, B: 0.5 µm; C: 2 µm.*

Ultrastructural signs of autophagy activation, such as sarcoplasmic multilayered membrane vesicles (the autophagosomes) engulfed with amorphous dense materials or mitochondria, were observed in the MDC1A muscle tissue (Fig. 2A). At IEM, LC3 was localized in large phagophores containing mitochondrial remnants (Fig. 2B). Autophagosomes engulfed with whorls of endoplasmic reticulum (ER) membranes and damaged mitochondria were also observed in the NM perinuclear areas where dilated cisterns of ER appeared to expand to include sarcoplasmic material (Fig. 2C). Vacuolar degeneration typical of autophagic cell death and nuclear condensation typical of apoptosis and autosis were not observed at light and electron microscope in the biopsies, while necrotic (pale, swollen, or myophagocytosed) myofibers were more frequently seen in MDC1A muscle tissue than the other neuromyopathies (Table 1). WB analysis was carried out and revealed relatively higher LC3 and p62 expression in MDC1A than NM, IMNM, and DMD patients (Suppl. Fig. 2). Altogether, the morphometric results suggest an active autophagy pathway in which p62 and cargo are degraded and do not accumulate in MDC1A, whereas in the other infantile neuromyopathies both LC3 and p62 seem to accumulate. Only in the MDC1A patient there was an excessive prevalence of type 2A myofibers (61.1%) calculated on ATPase stainings (pH 4.3, 4.6, 9.4; Suppl. Fig. 3; Table 1), even if myofiber sizes of all types appeared within the normal range, as compared with previously published data.[13]

## Discussion

In this pilot study, by IHC, IEM, and WB, we found hyperexpression of LC3, a marker of active autophagy, in MDC1A-affected muscle tissue. We compared this MDC1A-affected muscle tissue with those obtained for diagnostic purposes from other children affected by different neuromyopathies, NM10, SMA 0, DMD, from an adult subject affected by IMNM used as positive control,[1,8] and from an apparently healthy subject. The molecular activation of autophagy in MDC1A depends on intracellular signaling of LAMA2 via integrin binding involving the Akt and FOXO3 transcription factors, which inhibits autophagy.[7] In the MDC1A mouse model, the lack of LAMA2 is associated with excessive autophagy, Akt/PKB inhibition, and muscle degeneration.[7] Administration of 3-methyladenine (3-MA), an inhibitor of the autophagosome organizer Vps34, was sufficient to ameliorate the muscle morphology, locomotion and lifespan of the affected mice.[7]

In the MDC1A-affected muscle tissue, other autophagy markers were also expressed, namely Beclin-1 and ATG5, together with p62/SQSTM1, indicating an activated degrading flux in myofibers. Abundant p62/SQSTM1 accumulation seems prominent in DMD-affected myofibers than the other infantile neuromyopathies, at a level similar to that of adult IMNM. p62 accumulation could reveal impaired autophagy or lysosomal dysfunction, rather than abnormal activation.[14] In fact, p62 accumulation has also been reported in the prototypical example of autophagy flux impairment in a muscle disorder, namely glycogen storage disease type II due to a defect in the lysosomal enzyme acid α-glucosidase.[15] The shuttle molecule p62 has many roles in cellular processes, including selective autophagy of ubiquitinated cargos, cell survival, cell death, oxidative stress, DNA repair and inflammation.[16]

This acts as substrate of caspase 6 and 8,[17] promotes fully activated cullin-3-modified caspase 8,[16] and plays an important role in childhood-onset neurodegeneration,[18] through damaged mitochondria clustering in the perinuclear region of cultured HeLa cells.[19] At light and electron microscopy, morphological analysis of MDC1A and NEM10 demonstrates the presence of necrosis but the absence of autophagic cell death characterized by the presence of many vacuoles and autolysis with nuclear membrane shrinkage.[20] The increase of autophagosome density observed in the analyzed infantile neuromyopathies can be interpreted as either activation or impairment of autophagy. In fact, autophagy impairment causes accumulation of damaged organelles and autophagosomes as already found in DMD and mdx mice, a model of DMD disease, with persistent phosphorylation of Akt and mammalian target of rapamycin (mTOR) and corresponding downregulation of the autophagy-inducing genes LC3, Atg12, Gabarapl1 and Bnip3.[21] Also in SMA mouse gastrocnemius, lower levels of LC3II, Beclin-1, and p62 proteins were observed in the pre-symptomatic stage.[22] NMs have not been previously associated with altered autophagy. This study also provides preliminary evidence of autophagosome accumulation in myofibers of a NEM10-affected patient. In addition, autophagy flux impairment could increase unwanted antigen presentation of muscular neoepitopes and/or secretion of phagocytic “eat me” signals to macrophages.[8,9,23] This hypothesis is not corroborated by our observation of scattered MHC-II+ cells not preferentially associated with LC3+ or p62+ puncta. In addition, fast-twitching type II myofibers are mostly affected by impaired autophagy,[24] but could better survive increased activation of autophagy. The primary limitation of our study is the heterogeneous single cases of exceptionally rare neuromyophaties analyzed and, hence static morphologic assessment of autophagy instead of appropriate dynamic flux analysis. Nevertheless, our study focuses on two main autophagy markers, LC3 and p62, providing evidence that increased LC3 protein correlates with autophagy activity whereas p62 correlates with autophagy impairment.

In conclusion, our pilot study shows, in an MDC1A-affected newborn, upregulated LC3b, myofiber expression of other autophagy markers (Beclin-1, ATG5), and the presence of autophagosomes frequently internalizing concentric whorls of ER membranes and damaged mitochondria in the perinuclear areas. These results suggest that increased autophagy characterizes MDC1A pathogenesis, confirming the observations made in the dy3k/dy3k animal model of MDC1A.7 In DMD, NEM10, and SMA the accumulation of p62+ puncta suggests an autophagy impairment.

## List of acronyms

Akt - serine/threonine kinase
ATG5 - Autophagy-related 5
ATPase - adenosine triphosphatase
Bnip3 - Bcl-2/adenovirus E1B 19kDa interacting protein 3
CK - creatine kinase
CM - control muscle
CMDs - congenital muscular dystrophies
CTRL - control
DMD - Duchenne muscular dystrophy
DNA - deoxyribonucleic acid
dy3k/dy3k - merosin-deficient congenital muscular dystrophy 1A mouse model
ER - endoplasmic reticulum
Fig. - figure
FOXO3 - Forkhead box O3 transcription factor
Gabarapl1 - GABA type A receptor associated protein like 1
HB - healthy babies
HMGCR - 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
LAMA2 - laminin-α2
IEM - immuno-electron microscopy
IHC - immunohistochemistry
IMNM - immune-mediated necrotizing myopathy
LC3 - microtubule-associated protein 1A/1B-light chain 3
MDC1A - merosin-deficient congenital muscular dystrophy 1A
Mdx - mouse strain with a point mutation in its DMD gene
MHC-II - major histocompatibility complex type II
MRI - magnetic resonance imaging
mTOR - mammalian target of rapamycin
NM - Nemaline myopathy
p62/SQSTM1 - polyubiquitin-binding protein p62/ Sequestosome 1
PI3K - Phosphoinositide 3-kinases
PKB - Protein kinase B
SMA 0 - Spinal muscular atrophy type 0
TEM - Transmission electron microscopy;
Vps34 - vacuolar protein sorting 34
WB - western blotting

## Contributions of Authors

FG made confocal microscopy observation, quantification analyses and wrote the first draft of the manuscript; MM, RR, NR, SZ, TA, NL, FG contributed to conception and design of the research, performed the IHC, IEM, WB experiments, analyzed, and interpreted the data; MM, NR, NL: have contributed to drafting the manuscript; LDC, AR, ME, AB, EG, MR, DV made critical revision of the article. All Authors have read and approved all aspect of the work. All authors read and approved the final manuscript.

## Acknowledgments

We would like to thank M.V.C. Pragnell, BA, for linguistic help and Michelina de Giorgis and Franco Fumai for technical help. We apologize to colleagues whose studies were not cited owing to space restrictions. Partly presented at the XVIII Congress of the Italian Association of Myology, June 2018, Genova, Italy.

## Funding

ork was supported by the Italian Ministry of Education, University and Research (MIUR, Finanziamento delle attività base di ricerca 2017 to FG).

## Conflict of Interest

All Authors are aware of the contents, do not declare any financial interests/arrangements with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of this manuscript.

## Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## Corresponding Author

Francesco Girolamo, Department of Translational Biomedicine and Neuroscience, University of Bari "Aldo Moro", Unit of Human Anatomy and Histology, Piazza Giulio Cesare, 11, 70124, Bari, Italy. Phone: +390805478352.
ORCID iD: *0000-0001- 8706-3386*
Email: francesco.girolamo@uniba.it

*E-mails and ORCID iD of co-authors*

*Mariangela Mastrapasqua:* *mariangela.mastrapasqua@uniba.it*
*ORCID iD: 0009-0006-1011-6340*
*Roberta Rossi: roberta.rossi300675@libero.it*
*ORCID iD 0000-0003-4586-9044*
*Lucrezia De Cosmo: lucrezia.decosmo@asl.taranto.it*
*ORCID iD: 0009-0005-8015-0977*
*Annalisa Resta: lisaresta84@gmail.com*
*ORCID iD: None*
*Mariella Errede: mariella.errede@uniba.it*
*ORCID iD 0000-0002-8163-2338*
*Antonella Bizzoca: antonella.bizzoca@uniba.it*
*ORCID iD: 0000-0002-9094-0295*
*Stefania Zampatti: s.zampatti@hsantalucia.it*
*ORCID iD: 0000-0003-2502-7157*
*Nicoletta Resta: nicoletta.resta@uniba.it*
*ORCID iD 0000-0002-8163-2338*
*Emiliano Giardina: emiliano.giardina@uniroma2.it*
*ORCID iD: 0000-0002-2741-5009*
*Maddalena Ruggieri: maddalena.ruggieri@uniba.it*
*ORCID iD 0000-0001-7419-570X*
*Daniela Virgintino: daniela.virgintino@uniba.it*
*ORCID iD: 0000-0003-1041-5341*
*Tiziana Annese: annese@lum.it*
*ORCID iD 0000-0002-7752-0368*
*Nicola Laforgia: nicola.laforgia@uniba.it*
*ORCID iD: 0000-0002-4610-1216*

## References

1. Dubowitz V. 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14-16 May 1993. Neuromuscul Disord. 1994 Jan;4(1):75-81. doi: 10.1016/0960-8966(94)90051-5. PMID: 8173355.
2. Graziano A, Bianco F, D'Amico A, Moroni I, Messina S, Bruno C, Pegoraro E, Mora M, Astrea G, Magri F, Comi GP, Berardinelli A, Moggio M, Morandi L, Pini A, Petillo R, Tasca G, Monforte M, Minetti C, Mongini T, Ricci E, Gorni K, Battini R, Villanova M, Politano L, Gualandi F, Ferlini A, Muntoni F, Santorelli FM, Bertini E, Pane M, Mercuri E. Prevalence of congenital muscular dystrophy in Italy: a population study. Neurology. 2015 Mar 3;84(9):904-11. doi: 10.1212/WNL.0000000000001303. Epub 2015 Feb 4. PMID: 25653289; PMCID: PMC4351663.
3. Tomé FM, Evangelista T, Leclerc A, Sunada Y, Manole E, Estournet B, Barois A, Campbell KP, Fardeau M. Congenital muscular dystrophy with merosin deficiency. C R Acad Sci III. 1994 Apr;317(4):351-7. PMID: 8000914.
4. Zambon AA, Muntoni F. Congenital muscular dystrophies: What is new? Neuromuscul Disord. 2021 Oct;31(10):931-942. doi: 10.1016/j.nmd.2021.07.009. Epub 2021 Jul 28. PMID: 34470717.
5. Helbling-Leclerc A, Zhang X, Topaloglu H, Cruaud C, Tesson F, Weissenbach J, Tomé FM, Schwartz K, Fardeau M, Tryggvason K, et al. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. Nat Genet. 1995 Oct;11(2):216-8. doi: 10.1038/ng1095-216. PMID: 7550355.
6. Huang K, Bi FF, Yang H. A Systematic Review and Meta-Analysis of the Prevalence of Congenital Myopathy. Front Neurol. 2021 Nov 2;12:761636. doi: 10.3389/fneur.2021.761636. Erratum in: Front Neurol. 2022 Feb 14;13:857959. PMID: 34795634; PMCID: PMC8592924.
7. Carmignac V, Svensson M, Körner Z, Elowsson L, Matsumura C, Gawlik KI, Allamand V, Durbeej M. Autophagy is increased in laminin α2 chain-deficient muscle and its inhibition improves muscle morphology in a mouse model of MDC1A. Hum Mol Genet. 2011 Dec 15;20(24):4891-902. doi: 10.1093/hmg/ddr427. Epub 2011 Sep 14. PMID: 21920942.
8. Girolamo F, Lia A, Annese T, Giannini M, Amati A, D'Abbicco D, Tampoia M, Virgintino D, Ribatti D, Serlenga L, Iannone F, Trojano M. Autophagy markers LC3 and p62 accumulate in immune-mediated necrotizing myopathy. Muscle Nerve. 2019 Sep;60(3):315-327. doi: 10.1002/mus.26608. Epub 2019 Jun 21. PMID: 31172530.
9. Girolamo F, Lia A, Amati A, Strippoli M, Coppola C, Virgintino D, Roncali L, Toscano A, Serlenga L,

Trojano M. Overexpression of autophagic proteins in the skeletal muscle of sporadic inclusion body myositis. Neuropathol Appl Neurobiol. 2013 Dec;39(7):736-49. doi: 10.1111/nan.12040. PMID: 23452291.

10. Lia A, Annese T, Fornaro M, Giannini M, D'Abbicco D, Errede M, Lorusso L, Amati A, Tampoia M, Trojano M, Virgintino D, Ribatti D, Serlenga L, Iannone F, Girolamo F. Perivascular and endomysial macrophages expressing VEGF and CXCL12 promote angiogenesis in anti-HMGCR immune-mediated necrotizing myopathy. Rheumatology (Oxford). 2022 Aug 3;61(8):3448-3460. doi: 10.1093/rheumatology/keab900. PMID: 34864921.
11. Pontrelli P, Oranger A, Barozzino M, Divella C, Conserva F, Fiore MG, Rossi R, Papale M, Castellano G, Simone S, Laviola L, Giorgino F, Piscitelli D, Gallone A, Gesualdo L. Deregulation of autophagy under hyperglycemic conditions is dependent on increased lysine 63 ubiquitination: a candidate mechanism in the progression of diabetic nephropathy. J Mol Med (Berl). 2018 Jul;96(7):645-659. doi: 10.1007/s00109-018-1656-3. Epub 2018 May 27. PMID: 29806072.
12. Desantis S, Accogli G, Burk J, Zizza S, Mastrodonato M, Francioso EG, Rossi R, Crovace A, Resta L. Ultrastructural characteristics of ovine bone marrow-derived mesenchymal stromal cells cultured with a silicon stabilized tricalcium phosphate bioceramic. Microsc Res Tech. 2017 Nov;80(11):1189-1198. doi: 10.1002/jemt.22916. Epub 2017 Aug 11. PMID: 28799674.
13. Bennington JL, Krupp M. Morphometric analysis of muscle. In: Muscle pathology, ed. Heffner, Churchill Livingstone, New York, 1984: p. 43-71.
14. Zhao T, Zheng T, Yu H, Hu BH, Hu B, Ma P, Yang Y, Yang N, Hu J, Cao T, Chen G, Yan B, Peshoff M, Hatzoglou M, Geng R, Li B, Zheng QY. Autophagy impairment as a key feature for acetaminophen-induced ototoxicity. Cell Death Dis. 2021 Jan 4;12(1):3. doi: 10.1038/s41419-020-03328-6. PMID: 33414397; PMCID: PMC7791066.
15. Nascimbeni AC, Fanin M, Masiero E, Angelini C, Sandri M. Impaired autophagy contributes to muscle atrophy in glycogen storage disease type II patients. Autophagy. 2012 Nov;8(11):1697-700. doi: 10.4161/auto.21691. Epub 2012 Aug 31. PMID: 22940840; PMCID: PMC3494606.
16. Fan L, Yin S, Zhang E, Hu H. Role of p62 in the regulation of cell death induction. Apoptosis. 2018 Apr;23(3-4):187-193. doi: 10.1007/s10495-018-1445-z. PMID: 29480462.
17. Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, Abdellatif M, Abdoli A, Abel S, Abeliovich H, Abildgaard MH, Abudu YP, Acevedo-Arozena A, Adamopoulos IE, Adeli K, Adolph TE, Adornetto A, Aflaki E, Agam G, Agarwal A, Aggarwal BB, Agnello M, Agostinis P, Agrewala JN, Agrotis A, Aguilar PV, Ahmad ST, Ahmed ZM, Ahumada-Castro U, Aits S, Aizawa S, Akkoc Y, Akoumianaki T, Akpinar HA, Al-Abd AM, Al-Akra L, Al-Gharaibeh A, Alaoui-Jamali MA, Alberti S, Alcocer-Gómez E, Alessandri C, Ali M, Alim Al-Bari MA, Aliwaini S, Alizadeh J, Almacellas E, Almasan A, Alonso A, Alonso GD, Altan-Bonnet N, Altieri DC, Álvarez ÉMC, Alves S, Alves da Costa C, Alzaharna MM, Amadio M, Amantini C, Amaral C, Ambrosio S, Amer AO, Ammanathan V, An Z, Andersen SU, Andrabi SA, Andrade-Silva M, Andres AM, Angelini S, Ann D, Anozie UC, Ansari MY, Antas P, Antebi A, Antón Z, Anwar T, Apetoh L, Apostolova N, Araki T, Araki Y, Arasaki K, Araújo WL, Araya J, Arden C, Arévalo MA, Arguelles S, Arias E, Arikkath J, Arimoto H, Ariosa AR, Armstrong-James D, Arnauné-Pelloquin L, Aroca A, Arroyo DS, Arsov I, Artero R, Asaro DML, Aschner M, Ashrafizadeh M, Ashur-Fabian O, Atanasov AG, Au AK, Auberger P, Auner HW, Aurelian L, Autelli R, Avagliano L, Ávalos Y, Aveic S, Aveleira CA, Avin-Wittenberg T, Aydin Y, Ayton S, Ayyadevara S, Azzopardi M, Baba M, Backer JM, Backues SK, Bae DH, Bae ON, Bae SH, Baehrecke EH, Baek A, Baek SH, Baek SH, Bagetta G, Bagniewska-Zadworna A, Bai H, Bai J, Bai X, Bai Y, Bairagi N, Baksi S, Balbi T, Baldari CT, Balduini W, Ballabio A, Ballester M, Balazadeh S, Balzan R, Bandopadhyay R, Banerjee S, Banerjee S, Bánréti Á, Bao Y, Baptista MS, Baracca A, Barbati C, Bargiela A, Barilà D, Barlow PG, Barmada SJ, Barreiro E, Barreto GE, Bartek J, Bartel B, Bartolome A, Barve GR, Basagoudanavar SH, Bassham DC, Bast RC Jr, Basu A, Batoko H, Batten I, Baulieu EE, Baumgarner BL, Bayry J, Beale R, Beau I, Beaumatin F, Bechara LRG, Beck GR Jr, Beers MF, Begun J, Behrends C, Behrens GMN, Bei R, Bejarano E, Bel S, Behl C, Belaid A, Belgareh-Touzé N, Bellarosa C, Belleudi F, Belló Pérez M, Bello-Morales R, Beltran JSO, Beltran S, Benbrook DM, Bendorius M, Benitez BA, Benito-Cuesta I, Bensalem J, Berchtold MW, Berezowska S, Bergamaschi D, Bergami M, Bergmann A, Berliocchi L, Berlioz-Torrent C, Bernard A, Berthoux L, Besirli CG, Besteiro S, Betin VM, Beyaert R, Bezbradica JS, Bhaskar K, Bhatia-Kissova I, Bhattacharya R, Bhattacharya S, Bhattacharyya S, Bhuiyan MS, Bhutia SK, Bi L, Bi X, Biden TJ, Bijian K, Billes VA, Binart N, Bincoletto C, Birgisdottir AB, Bjorkoy G, Blanco G, Blas-Garcia A, Blasiak J, Blomgran R, Blomgren K, Blum JS, Boada-Romero E, Boban M, Boesze-Battaglia K, Boeuf P, Boland B, Bomont P, Bonaldo P, Bonam SR, Bonfili L, Bonifacino JS, Boone BA, Bootman MD, Bordi M, Borner C, Bornhauser BC, Borthakur G, Bosch J, Bose S,

Botana LM, Botas J, Boulanger CM, Boulton ME, Bourdenx M, Bourgeois B, Bourke NM, Bousquet G, Boya P, Bozhkov PV, Bozi LHM, Bozkurt TO, Brackney DE, Brandts CH, Braun RJ, Braus GH, Bravo-Sagua R, Bravo-San Pedro JM, Brest P, Bringer MA, Briones-Herrera A, Broaddus VC, Brodersen P, Brodsky JL, Brody SL, Bronson PG, Bronstein JM, Brown CN, Brown RE, Brum PC, Brumell JH, Brunetti-Pierri N, Bruno D, Bryson-Richardson RJ, Bucci C, Buchrieser C, Bueno M, Buitrago-Molina LE, Buraschi S, Buch S, Buchan JR, Buckingham EM, Budak H, Budini M, Bultynck G, Burada F, Burgoyne JR, Burón MI, Bustos V, Büttner S, Butturini E, Byrd A, Cabas I, Cabrera-Benitez S, Cadwell K, Cai J, Cai L, Cai Q, Cairó M, Calbet JA, Caldwell GA, Caldwell KA, Call JA, Calvani R, Calvo AC, Calvo-Rubio Barrera M, Camara NO, Camonis JH, Camougrand N, Campanella M, Campbell EM, Campbell-Valois FX, Campello S, Campesi I, Campos JC, Camuzard O, Cancino J, Candido de Almeida D, Canesi L, Caniggia I, Canonico B, Cantí C, Cao B, Caraglia M, Caramés B, Carchman EH, Cardenal-Muñoz E, Cardenas C, Cardenas L, Cardoso SM, Carew JS, Carle GF, Carleton G, Carloni S, Carmona-Gutierrez D, Carneiro LA, Carnevali O, Carosi JM, Carra S, Carrier A, Carrier L, Carroll B, Carter AB, Carvalho AN, Casanova M, Casas C, Casas J, Cassioli C, Castillo EF, Castillo K, Castillo-Lluva S, Castoldi F, Castori M, Castro AF, Castro-Caldas M, Castro-Hernandez J, Castro-Obregon S, Catz SD, Cavadas C, Cavaliere F, Cavallini G, Cavinato M, Cayuela ML, Cebollada Rica P, Cecarini V, Cecconi F, Cechowska-Pasko M, Cenci S, Ceperuelo-Mallafré V, Cerqueira JJ, Cerutti JM, Cervia D, Cetintas VB, Cetrullo S, Chae HJ, Chagin AS, Chai CY, Chakrabarti G, Chakrabarti O, Chakraborty T, Chakraborty T, Chami M, Chamilos G, Chan DW, Chan EYW, Chan ED, Chan HYE, Chan HH, Chan H, Chan MTV, Chan YS, Chandra PK, Chang CP, Chang C, Chang HC, Chang K, Chao J, Chapman T, Charlet-Berguerand N, Chatterjee S, Chaube SK, Chaudhary A, Chauhan S, Chaum E, Checler F, Cheetham ME, Chen CS, Chen GC, Chen JF, Chen LL, Chen L, Chen L, Chen M, Chen MK, Chen N, Chen Q, Chen RH, Chen S, Chen W, Chen W, Chen XM, Chen XW, Chen X, Chen Y, Chen YG, Chen Y, Chen Y, Chen YJ, Chen YQ, Chen ZS, Chen Z, Chen ZH, Chen ZJ, Chen Z, Cheng H, Cheng J, Cheng SY, Cheng W, Cheng X, Cheng XT, Cheng Y, Cheng Z, Chen Z, Cheong H, Cheong JK, Chernyak BV, Cherry S, Cheung CFR, Cheung CHA, Cheung KH, Chevet E, Chi RJ, Chiang AKS, Chiaradonna F, Chiarelli R, Chiariello M, Chica N, Chiocca S, Chiong M, Chiou SH, Chiramel AI, Chiurchiù V, Cho DH, Choe SK, Choi AMK, Choi ME, Choudhury KR, Chow NS, Chu CT, Chua JP, Chua JJE, Chung H, Chung KP, Chung S, Chung SH, Chung YL, Cianfanelli V, Ciechomska IA, Cifuentes M, Cinque L, Cirak S, Cirone M, Clague MJ, Clarke R, Clementi E, Coccia EM, Codogno P, Cohen E, Cohen MM, Colasanti T, Colasuonno F, Colbert RA, Colell A, Čolić M, Coll NS, Collins MO, Colombo MI, Colón-Ramos DA, Combaret L, Comincini S, Cominetti MR, Consiglio A, Conte A, Conti F, Contu VR, Cookson MR, Coombs KM, Coppens I, Corasaniti MT, Corkery DP, Cordes N, Cortese K, Costa MDC, Costantino S, Costelli P, Coto-Montes A, Crack PJ, Crespo JL, Criollo A, Crippa V, Cristofani R, Csizmadia T, Cuadrado A, Cui B, Cui J, Cui Y, Cui Y, Culetto E, Cumino AC, Cybulsky AV, Czaja MJ, Czuczwar SJ, D'Adamo S, D'Amelio M, D'Arcangelo D, D'Lugos AC, D'Orazi G, da Silva JA, Dafsari HS, Dagda RK, Dagdas Y, Daglia M, Dai X, Dai Y, Dai Y, Dal Col J, Dalhaimer P, Dalla Valle L, Dallenga T, Dalmasso G, Damme M, Dando I, Dantuma NP, Darling AL, Das H, Dasarathy S, Dasari SK, Dash S, Daumke O, Dauphinee AN, Davies JS, Dávila VA, Davis RJ, Davis T, Dayalan Naidu S, De Amicis F, De Bosscher K, De Felice F, De Franceschi L, De Leonibus C, de Mattos Barbosa MG, De Meyer GRY, De Milito A, De Nunzio C, De Palma C, De Santi M, De Virgilio C, De Zio D, Debnath J, DeBosch BJ, Decuypere JP, Deehan MA, Deflorian G, DeGregori J, Dehay B, Del Rio G, Delaney JR, Delbridge LMD, Delorme-Axford E, Delpino MV, Demarchi F, Dembitz V, Demers ND, Deng H, Deng Z, Dengjel J, Dent P, Denton D, DePamphilis ML, Der CJ, Deretic V, Descoteaux A, Devis L, Devkota S, Devuyst O, Dewson G, Dharmasivam M, Dhiman R, di Bernardo D, Di Cristina M, Di Domenico F, Di Fazio P, Di Fonzo A, Di Guardo G, Di Guglielmo GM, Di Leo L, Di Malta C, Di Nardo A, Di Rienzo M, Di Sano F, Diallinas G, Diao J, Diaz-Araya G, Díaz-Laviada I, Dickinson JM, Diederich M, Dieudé M, Dikic I, Ding S, Ding WX, Dini L, Dinić J, Dinic M, Dinkova-Kostova AT, Dionne MS, Distler JHW, Diwan A, Dixon IMC, Djavaheri-Mergny M, Dobrinski I, Dobrovinskaya O, Dobrowolski R, Dobson RCJ, Đokić J, Dokmeci Emre S, Donadelli M, Dong B, Dong X, Dong Z, Dorn Ii GW, Dotsch V, Dou H, Dou J, Dowaidar M, Dridi S, Drucker L, Du A, Du C, Du G, Du HN, Du LL, du Toit A, Duan SB, Duan X, Duarte SP, Dubrovska A, Dunlop EA, Dupont N, Durán RV, Dwarakanath BS, Dyshlovoy SA, Ebrahimi-Fakhari D, Eckhart L, Edelstein CL, Efferth T, Eftekharpour E, Eichinger L, Eid N, Eisenberg T, Eissa NT, Eissa S, Ejarque M, El Andaloussi A, El-Hage N, El-Naggar S, Eleuteri AM, El-Shafey ES, Elgendy M, Eliopoulos AG, Elizalde MM, Elks PM, Elsasser HP, Elsherbiny ES, Emerling BM, Emre NCT, Eng CH, Engedal N, Engelbrecht AM, Engelsen AST, Enserink JM, Escalante R, Esclatine

A, Escobar-Henriques M, Eskelinen EL, Espert L, Eusebio MO, Fabrias G, Fabrizi C, Facchiano A, Facchiano F, Fadeel B, Fader C, Faesen AC, Fairlie WD, Falcó A, Falkenburger BH, Fan D, Fan J, Fan Y, Fang EF, Fang Y, Fang Y, Fanto M, Farfel-Becker T, Faure M, Fazeli G, Fedele AO, Feldman AM, Feng D, Feng J, Feng L, Feng Y, Feng Y, Feng W, Fenz Araujo T, Ferguson TA, Fernández ÁF, Fernandez-Checa JC, Fernández-Veledo S, Fernie AR, Ferrante AW Jr, Ferraresi A, Ferrari MF, Ferreira JCB, Ferro-Novick S, Figueras A, Filadi R, Filigheddu N, Filippi-Chiela E, Filomeni G, Fimia GM, Fineschi V, Finetti F, Finkbeiner S, Fisher EA, Fisher PB, Flamigni F, Fliesler SJ, Flo TH, Florance I, Florey O, Florio T, Fodor E, Follo C, Fon EA, Forlino A, Fornai F, Fortini P, Fracassi A, Fraldi A, Franco B, Franco R, Franconi F, Frankel LB, Friedman SL, Fröhlich LF, Frühbeck G, Fuentes JM, Fujiki Y, Fujita N, Fujiwara Y, Fukuda M, Fulda S, Furic L, Furuya N, Fusco C, Gack MU, Gaffke L, Galadari S, Galasso A, Galindo MF, Gallolu Kankanamalage S, Galluzzi L, Galy V, Gammoh N, Gan B, Ganley IG, Gao F, Gao H, Gao M, Gao P, Gao SJ, Gao W, Gao X, Garcera A, Garcia MN, Garcia VE, García-Del Portillo F, Garcia-Escudero V, Garcia-Garcia A, Garcia-Macia M, García-Moreno D, Garcia-Ruiz C, García-Sanz P, Garg AD, Gargini R, Garofalo T, Garry RF, Gassen NC, Gatica D, Ge L, Ge W, Geiss-Friedlander R, Gelfi C, Genschik P, Gentle IE, Gerbino V, Gerhardt C, Germain K, Germain M, Gewirtz DA, Ghasemipour Afshar E, Ghavami S, Ghigo A, Ghosh M, Giamas G, Giampietri C, Giatromanolaki A, Gibson GE, Gibson SB, Ginet V, Giniger E, Giorgi C, Girao H, Girardin SE, Giridharan M, Giuliano S, Giulivi C, Giuriato S, Giustiniani J, Gluschko A, Goder V, Goginashvili A, Golab J, Goldstone DC, Golebiewska A, Gomes LR, Gomez R, Gómez-Sánchez R, Gomez-Puerto MC, Gomez-Sintes R, Gong Q, Goni FM, González-Gallego J, Gonzalez-Hernandez T, Gonzalez-Polo RA, Gonzalez-Reyes JA, González-Rodríguez P, Goping IS, Gorbatyuk MS, Gorbunov NV, Görgülü K, Gorojod RM, Gorski SM, Goruppi S, Gotor C, Gottlieb RA, Gozes I, Gozuacik D, Graef M, Gräler MH, Granatiero V, Grasso D, Gray JP, Green DR, Greenhough A, Gregory SL, Griffin EF, Grinstaff MW, Gros F, Grose C, Gross AS, Gruber F, Grumati P, Grune T, Gu X, Guan JL, Guardia CM, Guda K, Guerra F, Guerri C, Guha P, Guillén C, Gujar S, Gukovskaya A, Gukovsky I, Gunst J, Günther A, Guntur AR, Guo C, Guo C, Guo H, Guo LW, Guo M, Gupta P, Gupta SK, Gupta S, Gupta VB, Gupta V, Gustafsson AB, Gutterman DD, H B R, Haapasalo A, Haber JE, Hać A, Hadano S, Hafrén AJ, Haidar M, Hall BS, Halldén G, Hamacher-Brady A, Hamann A, Hamasaki M, Han W, Hansen M, Hanson PI, Hao Z, Harada M, Harhaji-Trajkovic L, Hariharan N, Haroon N, Harris J, Hasegawa T, Hasima Nagoor N, Haspel JA, Haucke V, Hawkins WD, Hay BA, Haynes CM, Hayrabedyan SB, Hays TS, He C, He Q, He RR, He YW, He YY, Heakal Y, Heberle AM, Hejtmancik JF, Helgason GV, Henkel V, Herb M, Hergovich A, Herman-Antosiewicz A, Hernández A, Hernandez C, Hernandez-Diaz S, Hernandez-Gea V, Herpin A, Herreros J, Hervás JH, Hesselson D, Hetz C, Heussler VT, Higuchi Y, Hilfiker S, Hill JA, Hlavacek WS, Ho EA, Ho IHT, Ho PW, Ho SL, Ho WY, Hobbs GA, Hochstrasser M, Hoet PHM, Hofius D, Hofman P, Höhn A, Holmberg CI, Hombrebueno JR, Yi-Ren Hong CH, Hooper LV, Hoppe T, Horos R, Hoshida Y, Hsin IL, Hsu HY, Hu B, Hu D, Hu LF, Hu MC, Hu R, Hu W, Hu YC, Hu ZW, Hua F, Hua J, Hua Y, Huan C, Huang C, Huang C, Huang C, Huang C, Huang H, Huang K, Huang MLH, Huang R, Huang S, Huang T, Huang X, Huang YJ, Huber TB, Hubert V, Hubner CA, Hughes SM, Hughes WE, Humbert M, Hummer G, Hurley JH, Hussain S, Hussain S, Hussey PJ, Hutabarat M, Hwang HY, Hwang S, Ieni A, Ikeda F, Imagawa Y, Imai Y, Imbriano C, Imoto M, Inman DM, Inoki K, Iovanna J, Iozzo RV, Ippolito G, Irazoqui JE, Iribarren P, Ishaq M, Ishikawa M, Ishimwe N, Isidoro C, Ismail N, Issazadeh-Navikas S, Itakura E, Ito D, Ivankovic D, Ivanova S, Iyer AKV, Izquierdo JM, Izumi M, Jäättelä M, Jabir MS, Jackson WT, Jacobo-Herrera N, Jacomin AC, Jacquin E, Jadiya P, Jaeschke H, Jagannath C, Jakobi AJ, Jakobsson J, Janji B, Jansen-Dürr P, Jansson PJ, Jantsch J, Januszewski S, Jassey A, Jean S, Jeltsch-David H, Jendelova P, Jenny A, Jensen TE, Jessen N, Jewell JL, Ji J, Jia L, Jia R, Jiang L, Jiang Q, Jiang R, Jiang T, Jiang X, Jiang Y, Jimenez-Sanchez M, Jin EJ, Jin F, Jin H, Jin L, Jin L, Jin M, Jin S, Jo EK, Joffre C, Johansen T, Johnson GVW, Johnston SA, Jokitalo E, Jolly MK, Joosten LAB, Jordan J, Joseph B, Ju D, Ju JS, Ju J, Juárez E, Judith D, Juhász G, Jun Y, Jung CH, Jung SC, Jung YK, Jungbluth H, Jungverdorben J, Just S, Kaarniranta K, Kaasik A, Kabuta T, Kaganovich D, Kahana A, Kain R, Kajimura S, Kalamvoki M, Kalia M, Kalinowski DS, Kaludercic N, Kalvari I, Kaminska J, Kaminskyy VO, Kanamori H, Kanasaki K, Kang C, Kang R, Kang SS, Kaniyappan S, Kanki T, Kanneganti TD, Kanthasamy AG, Kanthasamy A, Kantorow M, Kapuy O, Karamouzis MV, Karim MR, Karmakar P, Katare RG, Kato M, Kaufmann SHE, Kauppinen A, Kaushal GP, Kaushik S, Kawasaki K, Kazan K, Ke PY, Keating DJ, Keber U, Kehrl JH, Keller KE, Keller CW, Kemper JK, Kenific CM, Kepp O, Kermorgant S, Kern A, Ketteler R, Keulers TG, Khalfin B, Khalil H, Khambu B, Khan SY, Khandelwal VKM, Khandia R, Kho W, Khobrekar NV, Khuansuwan S, Khundadze M, Killackey SA,

Kim D, Kim DR, Kim DH, Kim DE, Kim EY, Kim EK, Kim HR, Kim HS, Hyung-Ryong Kim, Kim JH, Kim JK, Kim JH, Kim J, Kim JH, Kim KI, Kim PK, Kim SJ, Kimball SR, Kimchi A, Kimmelman AC, Kimura T, King MA, Kinghorn KJ, Kinsey CG, Kirkin V, Kirshenbaum LA, Kiselev SL, Kishi S, Kitamoto K, Kitaoka Y, Kitazato K, Kitsis RN, Kittler JT, Kjaerulff O, Klein PS, Klopstock T, Klucken J, Knævelsrud H, Knorr RL, Ko BCB, Ko F, Ko JL, Kobayashi H, Kobayashi S, Koch I, Koch JC, Koenig U, Kögel D, Koh YH, Koike M, Kohlwein SD, Kocaturk NM, Komatsu M, König J, Kono T, Kopp BT, Korcsmaros T, Korkmaz G, Korolchuk VI, Korsnes MS, Koskela A, Kota J, Kotake Y, Kotler ML, Kou Y, Koukourakis MI, Koustas E, Kovacs AL, Kovács T, Koya D, Kozako T, Kraft C, Krainc D, Krämer H, Krasnodembskaya AD, Kretz-Remy C, Kroemer G, Ktistakis NT, Kuchitsu K, Kuenen S, Kuerschner L, Kukar T, Kumar A, Kumar A, Kumar D, Kumar D, Kumar S, Kume S, Kumsta C, Kundu CN, Kundu M, Kunnumakkara AB, Kurgan L, Kutateladze TG, Kutlu O, Kwak S, Kwon HJ, Kwon TK, Kwon YT, Kyrmizi I, La Spada A, Labonté P, Ladoire S, Laface I, Lafont F, Lagace DC, Lahiri V, Lai Z, Laird AS, Lakkaraju A, Lamark T, Lan SH, Landajuela A, Lane DJR, Lane JD, Lang CH, Lange C, Langel Ü, Langer R, Lapaquette P, Laporte J, LaRusso NF, Lastres-Becker I, Lau WCY, Laurie GW, Lavandero S, Law BYK, Law HK, Layfield R, Le W, Le Stunff H, Leary AY, Lebrun JJ, Leck LYW, Leduc-Gaudet JP, Lee C, Lee CP, Lee DH, Lee EB, Lee EF, Lee GM, Lee HJ, Lee HK, Lee JM, Lee JS, Lee JA, Lee JY, Lee JH, Lee M, Lee MG, Lee MJ, Lee MS, Lee SY, Lee SJ, Lee SY, Lee SB, Lee WH, Lee YR, Lee YH, Lee Y, Lefebvre C, Legouis R, Lei YL, Lei Y, Leikin S, Leitinger G, Lemus L, Leng S, Lenoir O, Lenz G, Lenz HJ, Lenzi P, León Y, Leopoldino AM, Leschczyk C, Leskelä S, Letellier E, Leung CT, Leung PS, Leventhal JS, Levine B, Lewis PA, Ley K, Li B, Li DQ, Li J, Li J, Li J, Li K, Li L, Li M, Li M, Li M, Li M, Li M, Li PL, Li MQ, Li Q, Li S, Li T, Li W, Li W, Li X, Li YP, Li Y, Li Z, Li Z, Li Z, Lian J, Liang C, Liang Q, Liang W, Liang Y, Liang Y, Liao G, Liao L, Liao M, Liao YF, Librizzi M, Lie PPY, Lilly MA, Lim HJ, Lima TRR, Limana F, Lin C, Lin CW, Lin DS, Lin FC, Lin JD, Lin KM, Lin KH, Lin LT, Lin PH, Lin Q, Lin S, Lin SJ, Lin W, Lin X, Lin YX, Lin YS, Linden R, Lindner P, Ling SC, Lingor P, Linnemann AK, Liou YC, Lipinski MM, Lipovšek S, Lira VA, Lisiak N, Liton PB, Liu C, Liu CH, Liu CF, Liu CH, Liu F, Liu H, Liu HS, Liu HF, Liu H, Liu J, Liu J, Liu J, Liu L, Liu L, Liu M, Liu Q, Liu W, Liu W, Liu XH, Liu X, Liu X, Liu X, Liu X, Liu Y, Liu Y, Liu Y, Liu Y, Liu Y, Livingston JA, Lizard G, Lizcano JM, Ljubojevic-Holzer S, LLeonart ME, Llobet-Navàs D, Llorente A, Lo CH, Lobato-Márquez D, Long Q, Long YC, Loos B, Loos JA, López MG, López-Doménech G, López-Guerrero JA, López-Jiménez AT, López-Pérez Ó, López-Valero I, Lorenowicz MJ, Lorente M, Lorincz P, Lossi L, Lotersztajn S, Lovat PE, Lovell JF, Lovy A, Lőw P, Lu G, Lu H, Lu JH, Lu JJ, Lu M, Lu S, Luciani A, Lucocq JM, Ludovico P, Luftig MA, Luhr M, Luis-Ravelo D, Lum JJ, Luna-Dulcey L, Lund AH, Lund VK, Lünemann JD, Lüningschrör P, Luo H, Luo R, Luo S, Luo Z, Luparello C, Lüscher B, Luu L, Lyakhovich A, Lyamzaev KG, Lystad AH, Lytvynchuk L, Ma AC, Ma C, Ma M, Ma NF, Ma QH, Ma X, Ma Y, Ma Z, MacDougald OA, Macian F, MacIntosh GC, MacKeigan JP, Macleod KF, Maday S, Madeo F, Madesh M, Madl T, Madrigal-Matute J, Maeda A, Maejima Y, Magarinos M, Mahavadi P, Maiani E, Maiese K, Maiti P, Maiuri MC, Majello B, Major MB, Makareeva E, Malik F, Mallilankaraman K, Malorni W, Maloyan A, Mammadova N, Man GCW, Manai F, Mancias JD, Mandelkow EM, Mandell MA, Manfredi AA, Manjili MH, Manjithaya R, Manque P, Manshian BB, Manzano R, Manzoni C, Mao K, Marchese C, Marchetti S, Marconi AM, Marcucci F, Mardente S, Mareninova OA, Margeta M, Mari M, Marinelli S, Marinelli O, Mariño G, Mariotto S, Marshall RS, Marten MR, Martens S, Martin APJ, Martin KR, Martin S, Martin S, Martín-Segura A, Martín-Acebes MA, Martin-Burriel I, Martin-Rincon M, Martin-Sanz P, Martina JA, Martinet W, Martinez A, Martinez A, Martinez J, Martinez Velazquez M, Martinez-Lopez N, Martinez-Vicente M, Martins DO, Martins JO, Martins WK, Martins-Marques T, Marzetti E, Masaldan S, Masclaux-Daubresse C, Mashek DG, Massa V, Massieu L, Masson GR, Masuelli L, Masyuk AI, Masyuk TV, Matarrese P, Matheu A, Matoba S, Matsuzaki S, Mattar P, Matte A, Mattoscio D, Mauriz JL, Mauthe M, Mauvezin C, Maverakis E, Maycotte P, Mayer J, Mazzoccoli G, Mazzoni C, Mazzulli JR, McCarty N, McDonald C, McGill MR, McKenna SL, McLaughlin B, McLoughlin F, McNiven MA, McWilliams TG, Mechta-Grigoriou F, Medeiros TC, Medina DL, Megeney LA, Megyeri K, Mehrpour M, Mehta JL, Meijer AJ, Meijer AH, Mejlvang J, Meléndez A, Melk A, Memisoglu G, Mendes AF, Meng D, Meng F, Meng T, Menna-Barreto R, Menon MB, Mercer C, Mercier AE, Mergny JL, Merighi A, Merkley SD, Merla G, Meske V, Mestre AC, Metur SP, Meyer C, Meyer H, Mi W, Mialet-Perez J, Miao J, Micale L, Miki Y, Milan E, Milczarek M, Miller DL, Miller SI, Miller S, Millward SW, Milosevic I, Minina EA, Mirzaei H, Mirzaei HR, Mirzaei M, Mishra A, Mishra N, Mishra PK, Misirkic Marjanovic M, Misasi R, Misra A, Misso G, Mitchell C, Mitou G, Miura T, Miyamoto S, Miyazaki M, Miyazaki M, Miyazaki T, Miyazawa

K, Mizushima N, Mogensen TH, Mograbi B, Mohammadinejad R, Mohamud Y, Mohanty A, Mohapatra S, Möhlmann T, Mohmmed A, Moles A, Moley KH, Molinari M, Mollace V, Møller AB, Mollereau B, Mollinedo F, Montagna C, Monteiro MJ, Montella A, Montes LR, Montico B, Mony VK, Monzio Compagnoni G, Moore MN, Moosavi MA, Mora AL, Mora M, Morales-Alamo D, Moratalla R, Moreira PI, Morelli E, Moreno S, Moreno-Blas D, Moresi V, Morga B, Morgan AH, Morin F, Morishita H, Moritz OL, Moriyama M, Moriyasu Y, Morleo M, Morselli E, Moruno-Manchon JF, Moscat J, Mostowy S, Motori E, Moura AF, Moustaid-Moussa N, Mrakovcic M, Muciño-Hernández G, Mukherjee A, Mukhopadhyay S, Mulcahy Levy JM, Mulero V, Muller S, Münch C, Munjal A, Munoz-Canoves P, Muñoz-Galdeano T, Münz C, Murakawa T, Muratori C, Murphy BM, Murphy JP, Murthy A, Myöhänen TT, Mysorekar IU, Mytych J, Nabavi SM, Nabissi M, Nagy P, Nah J, Nahimana A, Nakagawa I, Nakamura K, Nakatogawa H, Nandi SS, Nanjundan M, Nanni M, Napolitano G, Nardacci R, Narita M, Nassif M, Nathan I, Natsumeda M, Naude RJ, Naumann C, Naveiras O, Navid F, Nawrocki ST, Nazarko TY, Nazio F, Negoita F, Neill T, Neisch AL, Neri LM, Netea MG, Neubert P, Neufeld TP, Neumann D, Neutzner A, Newton PT, Ney PA, Nezis IP, Ng CCW, Ng TB, Nguyen HTT, Nguyen LT, Ni HM, Ní Cheallaigh C, Ni Z, Nicolao MC, Nicoli F, Nieto-Diaz M, Nilsson P, Ning S, Niranjan R, Nishimune H, Niso-Santano M, Nixon RA, Nobili A, Nobrega C, Noda T, Nogueira-Recalde U, Nolan TM, Nombela I, Novak I, Novoa B, Nozawa T, Nukina N, Nussbaum-Krammer C, Nylandsted J, O'Donovan TR, O'Leary SM, O'Rourke EJ, O'Sullivan MP, O'Sullivan TE, Oddo S, Oehme I, Ogawa M, Ogier-Denis E, Ogmundsdottir MH, Ogretmen B, Oh GT, Oh SH, Oh YJ, Ohama T, Ohashi Y, Ohmuraya M, Oikonomou V, Ojha R, Okamoto K, Okazawa H, Oku M, Oliván S, Oliveira JMA, Ollmann M, Olzmann JA, Omari S, Omary MB, Önal G, Ondrej M, Ong SB, Ong SG, Onnis A, Orellana JA, Orellana-Muñoz S, Ortega-Villaizan MDM, Ortiz-Gonzalez XR, Ortona E, Osiewacz HD, Osman AK, Osta R, Otegui MS, Otsu K, Ott C, Ottobrini L, Ou JJ, Outeiro TF, Oynebraten I, Ozturk M, Pagès G, Pahari S, Pajares M, Pajvani UB, Pal R, Paladino S, Pallet N, Palmieri M, Palmisano G, Palumbo C, Pampaloni F, Pan L, Pan Q, Pan W, Pan X, Panasyuk G, Pandey R, Pandey UB, Pandya V, Paneni F, Pang SY, Panzarini E, Papademetrio DL, Papaleo E, Papinski D, Papp D, Park EC, Park HT, Park JM, Park JI, Park JT, Park J, Park SC, Park SY, Parola AH, Parys JB, Pasquier A, Pasquier B, Passos JF, Pastore N, Patel HH, Patschan D, Pattingre S, Pedraza-Alva G, Pedraza-Chaverri J, Pedrozo Z, Pei G, Pei J, Peled-Zehavi H, Pellegrini JM, Pelletier J, Peñalva MA, Peng D, Peng Y, Penna F, Pennuto M, Pentimalli F, Pereira CM, Pereira GJS, Pereira LC, Pereira de Almeida L, Perera ND, Pérez-Lara Á, Perez-Oliva AB, Pérez-Pérez ME, Periyasamy P, Perl A, Perrotta C, Perrotta I, Pestell RG, Petersen M, Petrache I, Petrovski G, Pfirrmann T, Pfister AS, Philips JA, Pi H, Picca A, Pickrell AM, Picot S, Pierantoni GM, Pierdominici M, Pierre P, Pierrefite-Carle V, Pierzynowska K, Pietrocola F, Pietruczuk M, Pignata C, Pimentel-Muiños FX, Pinar M, Pinheiro RO, Pinkas-Kramarski R, Pinton P, Pircs K, Piya S, Pizzo P, Plantinga TS, Platta HW, Plaza-Zabala A, Plomann M, Plotnikov EY, Plun-Favreau H, Pluta R, Pocock R, Pöggeler S, Pohl C, Poirot M, Poletti A, Ponpuak M, Popelka H, Popova B, Porta H, Porte Alcon S, Portilla-Fernandez E, Post M, Potts MB, Poulton J, Powers T, Prahlad V, Prajsnar TK, Praticò D, Prencipe R, Priault M, Proikas-Cezanne T, Promponas VJ, Proud CG, Puertollano R, Puglielli L, Pulinilkunnil T, Puri D, Puri R, Puyal J, Qi X, Qi Y, Qian W, Qiang L, Qiu Y, Quadrilatero J, Quarleri J, Raben N, Rabinowich H, Ragona D, Ragusa MJ, Rahimi N, Rahmati M, Raia V, Raimundo N, Rajasekaran NS, Ramachandra Rao S, Rami A, Ramírez-Pardo I, Ramsden DB, Randow F, Rangarajan PN, Ranieri D, Rao H, Rao L, Rao R, Rathore S, Ratnayaka JA, Ratovitski EA, Ravanan P, Ravegnini G, Ray SK, Razani B, Rebecca V, Reggiori F, Régnier-Vigouroux A, Reichert AS, Reigada D, Reiling JH, Rein T, Reipert S, Rekha RS, Ren H, Ren J, Ren W, Renault T, Renga G, Reue K, Rewitz K, Ribeiro de Andrade Ramos B, Riazuddin SA, Ribeiro-Rodrigues TM, Ricci JE, Ricci R, Riccio V, Richardson DR, Rikihisa Y, Risbud MV, Risueño RM, Ritis K, Rizza S, Rizzuto R, Roberts HC, Roberts LD, Robinson KJ, Roccheri MC, Rocchi S, Rodney GG, Rodrigues T, Rodrigues Silva VR, Rodriguez A, Rodriguez-Barrueco R, Rodriguez-Henche N, Rodriguez-Rocha H, Roelofs J, Rogers RS, Rogov VV, Rojo AI, Rolka K, Romanello V, Romani L, Romano A, Romano PS, Romeo-Guitart D, Romero LC, Romero M, Roney JC, Rongo C, Roperto S, Rosenfeldt MT, Rosenstiel P, Rosenwald AG, Roth KA, Roth L, Roth S, Rouschop KMA, Roussel BD, Roux S, Rovere-Querini P, Roy A, Rozieres A, Ruano D, Rubinsztein DC, Rubtsova MP, Ruckdeschel K, Ruckenstuhl C, Rudolf E, Rudolf R, Ruggieri A, Ruparelia AA, Rusmini P, Russell RR, Russo GL, Russo M, Russo R, Ryabaya OO, Ryan KM, Ryu KY, Sabater-Arcis M, Sachdev U, Sacher M, Sachse C, Sadhu A, Sadoshima J, Safren N, Saftig P, Sagona AP, Sahay G, Sahebkar A, Sahin M, Sahin O, Sahni S, Saito N, Saito S, Saito T, Sakai R, Sakai Y, Sakamaki JI, Saksela K, Salazar G, Salazar-Degracia A, Salekdeh GH,

Saluja AK, Sampaio-Marques B, Sanchez MC, Sanchez-Alcazar JA, Sanchez-Vera V, Sancho-Shimizu V, Sanderson JT, Sandri M, Santaguida S, Santambrogio L, Santana MM, Santoni G, Sanz A, Sanz P, Saran S, Sardiello M, Sargeant TJ, Sarin A, Sarkar C, Sarkar S, Sarrias MR, Sarkar S, Sarmah DT, Sarparanta J, Sathyanarayan A, Sathyanarayanan R, Scaglione KM, Scatozza F, Schaefer L, Schafer ZT, Schaible UE, Schapira AHV, Scharl M, Schatzl HM, Schein CH, Scheper W, Scheuring D, Schiaffino MV, Schiappacassi M, Schindl R, Schlattner U, Schmidt O, Schmitt R, Schmidt SD, Schmitz I, Schmukler E, Schneider A, Schneider BE, Schober R, Schoijet AC, Schott MB, Schramm M, Schröder B, Schuh K, Schüller C, Schulze RJ, Schürmanns L, Schwamborn JC, Schwarten M, Scialo F, Sciarretta S, Scott MJ, Scotto KW, Scovassi AI, Scrima A, Scrivo A, Sebastian D, Sebti S, Sedej S, Segatori L, Segev N, Seglen PO, Seiliez I, Seki E, Selleck SB, Sellke FW, Selsby JT, Sendtner M, Senturk S, Seranova E, Sergi C, Serra-Moreno R, Sesaki H, Settembre C, Setty SRG, Sgarbi G, Sha O, Shacka JJ, Shah JA, Shang D, Shao C, Shao F, Sharbati S, Sharkey LM, Sharma D, Sharma G, Sharma K, Sharma P, Sharma S, Shen HM, Shen H, Shen J, Shen M, Shen W, Shen Z, Sheng R, Sheng Z, Sheng ZH, Shi J, Shi X, Shi YH, Shiba-Fukushima K, Shieh JJ, Shimada Y, Shimizu S, Shimozawa M, Shintani T, Shoemaker CJ, Shojaei S, Shoji I, Shravage BV, Shridhar V, Shu CW, Shu HB, Shui K, Shukla AK, Shutt TE, Sica V, Siddiqui A, Sierra A, Sierra-Torre V, Signorelli S, Sil P, Silva BJA, Silva JD, Silva-Pavez E, Silvente-Poirot S, Simmonds RE, Simon AK, Simon HU, Simons M, Singh A, Singh LP, Singh R, Singh SV, Singh SK, Singh SB, Singh S, Singh SP, Sinha D, Sinha RA, Sinha S, Sirko A, Sirohi K, Sivridis EL, Skendros P, Skirycz A, Slaninová I, Smaili SS, Smertenko A, Smith MD, Soenen SJ, Sohn EJ, Sok SPM, Solaini G, Soldati T, Soleimanpour SA, Soler RM, Solovchenko A, Somarelli JA, Sonawane A, Song F, Song HK, Song JX, Song K, Song Z, Soria LR, Sorice M, Soukas AA, Soukup SF, Sousa D, Sousa N, Spagnuolo PA, Spector SA, Srinivas Bharath MM, St Clair D, Stagni V, Staiano L, Stalnecker CA, Stankov MV, Stathopulos PB, Stefan K, Stefan SM, Stefanis L, Steffan JS, Steinkasserer A, Stenmark H, Sterneckert J, Stevens C, Stoka V, Storch S, Stork B, Strappazzon F, Strohecker AM, Stupack DG, Su H, Su LY, Su L, Suarez-Fontes AM, Subauste CS, Subbian S, Subirada PV, Sudhandiran G, Sue CM, Sui X, Summers C, Sun G, Sun J, Sun K, Sun MX, Sun Q, Sun Y, Sun Z, Sunahara KKS, Sundberg E, Susztak K, Sutovsky P, Suzuki H, Sweeney G, Symons JD, Sze SCW, Szewczyk NJ, Tabęcka-Łonczynska A, Tabolacci C, Tacke F, Taegtmeyer H, Tafani M, Tagaya M, Tai H, Tait SWG, Takahashi Y, Takats S, Talwar P, Tam C, Tam SY, Tampellini D, Tamura A, Tan CT, Tan EK, Tan YQ, Tanaka M, Tanaka M, Tang D, Tang J, Tang TS, Tanida I, Tao Z, Taouis M, Tatenhorst L, Tavernarakis N, Taylor A, Taylor GA, Taylor JM, Tchetina E, Tee AR, Tegeder I, Teis D, Teixeira N, Teixeira-Clerc F, Tekirdag KA, Tencomnao T, Tenreiro S, Tepikin AV, Testillano PS, Tettamanti G, Tharaux PL, Thedieck K, Thekkinghat AA, Thellung S, Thinwa JW, Thirumalaikumar VP, Thomas SM, Thomes PG, Thorburn A, Thukral L, Thum T, Thumm M, Tian L, Tichy A, Till A, Timmerman V, Titorenko VI, Todi SV, Todorova K, Toivonen JM, Tomaipitinca L, Tomar D, Tomas-Zapico C, Tomić S, Tong BC, Tong C, Tong X, Tooze SA, Torgersen ML, Torii S, Torres-López L, Torriglia A, Towers CG, Towns R, Toyokuni S, Trajkovic V, Tramontano D, Tran QG, Travassos LH, Trelford CB, Tremel S, Trougakos IP, Tsao BP, Tschan MP, Tse HF, Tse TF, Tsugawa H, Tsvetkov AS, Tumbarello DA, Tumtas Y, Tuñón MJ, Turcotte S, Turk B, Turk V, Turner BJ, Tuxworth RI, Tyler JK, Tyutereva EV, Uchiyama Y, Ugun-Klusek A, Uhlig HH, Ułamek-Kozioł M, Ulasov IV, Umekawa M, Ungermann C, Unno R, Urbe S, Uribe-Carretero E, Üstün S, Uversky VN, Vaccari T, Vaccaro MI, Vahsen BF, Vakifahmetoglu-Norberg H, Valdor R, Valente MJ, Valko A, Vallee RB, Valverde AM, Van den Berghe G, van der Veen S, Van Kaer L, van Loosdregt J, van Wijk SJL, Vandenberghe W, Vanhorebeek I, Vannier-Santos MA, Vannini N, Vanrell MC, Vantaggiato C, Varano G, Varela-Nieto I, Varga M, Vasconcelos MH, Vats S, Vavvas DG, Vega-Naredo I, Vega-Rubin-de-Celis S, Velasco G, Velázquez AP, Vellai T, Vellenga E, Velotti F, Verdier M, Verginis P, Vergne I, Verkade P, Verma M, Verstreken P, Vervliet T, Vervoorts J, Vessoni AT, Victor VM, Vidal M, Vidoni C, Vieira OV, Vierstra RD, Viganó S, Vihinen H, Vijayan V, Vila M, Vilar M, Villalba JM, Villalobo A, Villarejo-Zori B, Villarroya F, Villarroya J, Vincent O, Vindis C, Viret C, Viscomi MT, Visnjic D, Vitale I, Vocadlo DJ, Voitsekhovskaja OV, Volonté C, Volta M, Vomero M, Von Haefen C, Vooijs MA, Voos W, Vucicevic L, Wade-Martins R, Waguri S, Waite KA, Wakatsuki S, Walker DW, Walker MJ, Walker SA, Walter J, Wandosell FG, Wang B, Wang CY, Wang C, Wang C, Wang C, Wang CY, Wang D, Wang F, Wang F, Wang F, Wang G, Wang H, Wang H, Wang H, Wang HG, Wang J, Wang J, Wang J, Wang J, Wang K, Wang L, Wang L, Wang MH, Wang M, Wang N, Wang P, Wang P, Wang P, Wang P, Wang QJ, Wang Q, Wang QK, Wang QA, Wang WT, Wang W, Wang X, Wang X, Wang Y, Wang Y, Wang Y, Wang YY, Wang Y, Wang Y, Wang Y, Wang Y, Wang Z, Wang Z, Wang Z, Warnes G, Warnsmann V, Watada H,

Watanabe E, Watchon M, Wawrzyńska A, Weaver TE, Wegrzyn G, Wehman AM, Wei H, Wei L, Wei T, Wei Y, Weiergräber OH, Weihl CC, Weindl G, Weiskirchen R, Wells A, Wen RH, Wen X, Werner A, Weykopf B, Wheatley SP, Whitton JL, Whitworth AJ, Wiktorska K, Wildenberg ME, Wileman T, Wilkinson S, Willbold D, Williams B, Williams RSB, Williams RL, Williamson PR, Wilson RA, Winner B, Winsor NJ, Witkin SS, Wodrich H, Woehlbier U, Wollert T, Wong E, Wong JH, Wong RW, Wong VKW, Wong WW, Wu AG, Wu C, Wu J, Wu J, Wu KK, Wu M, Wu SY, Wu S, Wu SY, Wu S, Wu WKK, Wu X, Wu X, Wu YW, Wu Y, Xavier RJ, Xia H, Xia L, Xia Z, Xiang G, Xiang J, Xiang M, Xiang W, Xiao B, Xiao G, Xiao H, Xiao HT, Xiao J, Xiao L, Xiao S, Xiao Y, Xie B, Xie CM, Xie M, Xie Y, Xie Z, Xie Z, Xilouri M, Xu C, Xu E, Xu H, Xu J, Xu J, Xu L, Xu WW, Xu X, Xue Y, Yakhine-Diop SMS, Yamaguchi M, Yamaguchi O, Yamamoto A, Yamashina S, Yan S, Yan SJ, Yan Z, Yanagi Y, Yang C, Yang DS, Yang H, Yang HT, Yang H, Yang JM, Yang J, Yang J, Yang L, Yang L, Yang M, Yang PM, Yang Q, Yang S, Yang S, Yang SF, Yang W, Yang WY, Yang X, Yang X, Yang Y, Yang Y, Yao H, Yao S, Yao X, Yao YG, Yao YM, Yasui T, Yazdankhah M, Yen PM, Yi C, Yin XM, Yin Y, Yin Z, Yin Z, Ying M, Ying Z, Yip CK, Yiu SPT, Yoo YH, Yoshida K, Yoshii SR, Yoshimori T, Yousefi B, Yu B, Yu H, Yu J, Yu J, Yu L, Yu ML, Yu SW, Yu VC, Yu WH, Yu Z, Yu Z, Yuan J, Yuan LQ, Yuan S, Yuan SF, Yuan Y, Yuan Z, Yue J, Yue Z, Yun J, Yung RL, Zacks DN, Zaffagnini G, Zambelli VO, Zanella I, Zang QS, Zanivan S, Zappavigna S, Zaragoza P, Zarbalis KS, Zarebkohan A, Zarrouk A, Zeitlin SO, Zeng J, Zeng JD, Žerovnik E, Zhan L, Zhang B, Zhang DD, Zhang H, Zhang H, Zhang H, Zhang H, Zhang H, Zhang H, Zhang H, Zhang HL, Zhang J, Zhang J, Zhang JP, Zhang KYB, Zhang LW, Zhang L, Zhang L, Zhang L, Zhang L, Zhang M, Zhang P, Zhang S, Zhang W, Zhang X, Zhang XW, Zhang X, Zhang X, Zhang X, Zhang X, Zhang XD, Zhang Y, Zhang Y, Zhang Y, Zhang YD, Zhang Y, Zhang YY, Zhang Y, Zhang Z, Zhang Z, Zhang Z, Zhang Z, Zhang Z, Zhang Z, Zhao H, Zhao L, Zhao S, Zhao T, Zhao XF, Zhao Y, Zhao Y, Zhao Y, Zhao Y, Zheng G, Zheng K, Zheng L, Zheng S, Zheng XL, Zheng Y, Zheng ZG, Zhivotovsky B, Zhong Q, Zhou A, Zhou B, Zhou C, Zhou G, Zhou H, Zhou H, Zhou H, Zhou J, Zhou J, Zhou J, Zhou J, Zhou K, Zhou R, Zhou XJ, Zhou Y, Zhou Y, Zhou Y, Zhou ZY, Zhou Z, Zhu B, Zhu C, Zhu GQ, Zhu H, Zhu H, Zhu H, Zhu WG, Zhu Y, Zhu Y, Zhuang H, Zhuang X, Zientara-Rytter K, Zimmermann CM, Ziviani E, Zoladek T, Zong WX, Zorov DB, Zorzano A, Zou W, Zou Z, Zou Z, Zuryn S, Zwerschke W, Brand-Saberi B, Dong XC, Kenchappa CS, Li Z, Lin Y, Oshima S, Rong Y, Sluimer JC, Stallings CL, Tong CK. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)[1]. Autophagy. 2021 Jan;17(1):1-382. doi: 10.1080/15548627.2020.1797280. Epub 2021 Feb 8. PMID: 33634751; PMCID: PMC7996087.

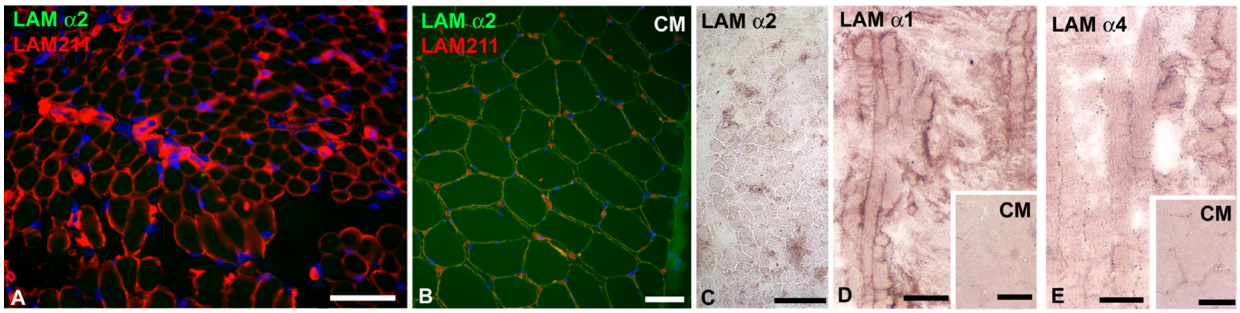
18. Haack TB, Ignatius E, Calvo-Garrido J, Iuso A, Isohanni P, Maffezzini C, Lönnqvist T, Suomalainen A, Gorza M, Kremer LS, Graf E, Hartig M, Berutti R, Paucar M, Svenningsson P, Stranneheim H, Brandberg G, Wedell A, Kurian MA, Hayflick SA, Venco P, Tiranti V, Strom TM, Dichgans M, Horvath R, Holinski-Feder E, Freyer C, Meitinger T, Prokisch H, Senderek J, Wredenberg A, Carroll CJ, Klopstock T. Absence of the Autophagy Adaptor SQSTM1/p62 Causes Childhood-Onset Neurodegeneration with Ataxia, Dystonia, and Gaze Palsy. Am J Hum Genet. 2016 Sep 1;99(3):735-743. doi: 10.1016/j.ajhg.2016.06.026. Epub 2016 Aug 18. PMID: 27545679; PMCID: PMC5010644.
19. Okatsu K, Saisho K, Shimanuki M, Nakada K, Shitara H, Sou YS, Kimura M, Sato S, Hattori N, Komatsu M, Tanaka K, Matsuda N. p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. Genes Cells. 2010 Aug;15(8):887-900. doi: 10.1111/j.1365-2443.2010.01426.x. Epub 2010 Jul 2. PMID: 20604804; PMCID: PMC2970908.
20. Liu Y, Levine B. Autosis and autophagic cell death: the dark side of autophagy. Cell Death Differ. 2015 Mar;22(3):367-76. doi: 10.1038/cdd.2014.143. Epub 2014 Sep 26. PMID: 25257169; PMCID: PMC4326571.
21. De Palma C, Morisi F, Cheli S, Pambianco S, Cappello V, Vezzoli M, Rovere-Querini P, Moggio M, Ripolone M, Francolini M, Sandri M, Clementi E. Autophagy as a new therapeutic target in Duchenne muscular dystrophy. Cell Death Dis. 2012 Nov 15;3(11):e418. doi: 10.1038/cddis.2012.159. PMID: 23152054; PMCID: PMC3542595.
22. Sansa A, Hidalgo I, Miralles MP, de la Fuente S, Perez-Garcia MJ, Munell F, Soler RM, Garcera A. Spinal Muscular Atrophy autophagy profile is tissue-dependent: differential regulation between muscle and motoneurons. Acta Neuropathol Commun. 2021 Jul 3;9(1):122. doi: 10.1186/s40478-021-01223-5. PMID: 34217376; PMCID: PMC8254901.
23. Boyle KB, Randow F. The role of 'eat-me' signals and autophagy cargo receptors in innate immunity. Curr Opin Microbiol. 2013 Jun;16(3):339-48. doi: 10.1016/j.mib.2013.03.010. Epub 2013 Apr 23. PMID: 23623150.
24. Wang C, Zhao B, Zhai J, Wang A, Cao N, Liao T, Su R, He L, Li Y, Pei X, Jia Y, Yue W. Clinical-

grade human umbilical cord-derived mesenchymal stem cells improved skeletal muscle dysfunction in age-associated sarcopenia mice. Cell Death Dis. 2023 May 12;14(5):321. doi: 10.1038/s41419-023-05843-8. PMID: 37173309; PMCID: PMC10182022.

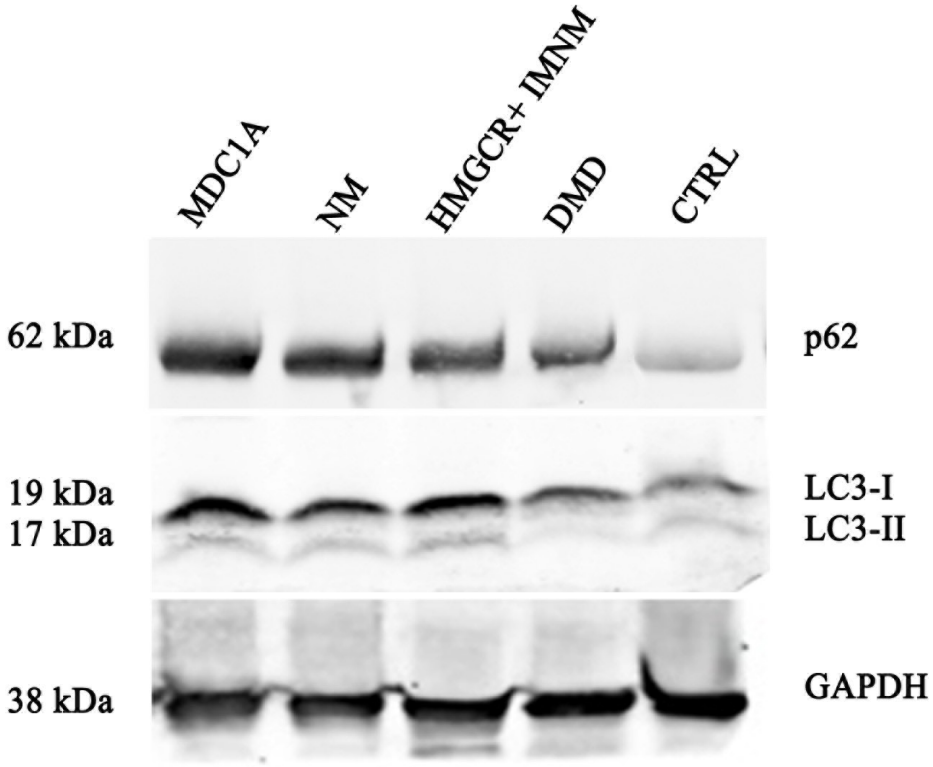
## Disclaimer

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

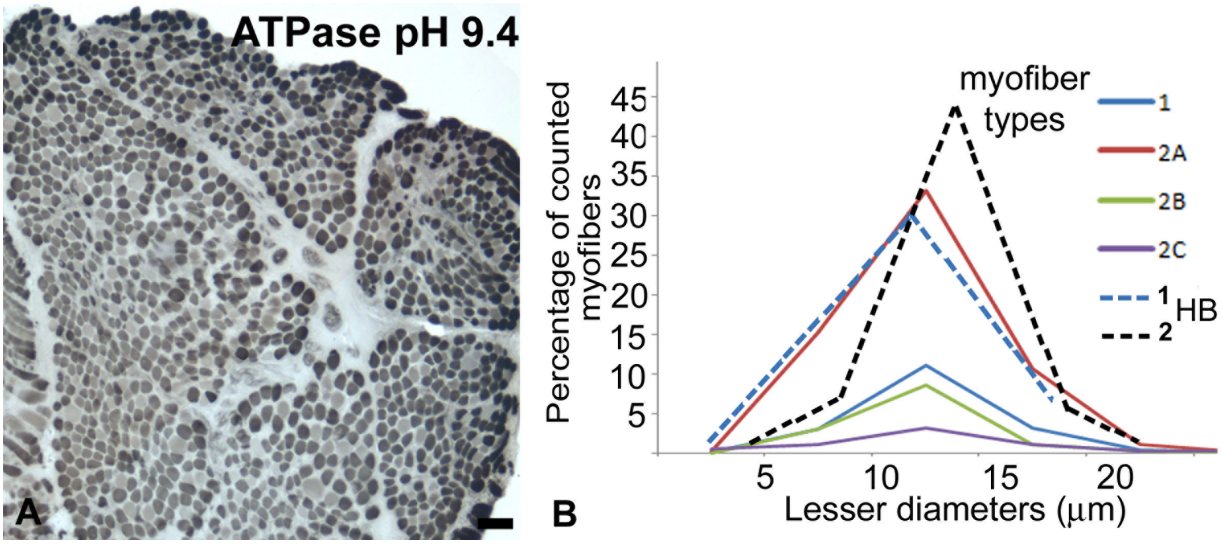
Submission: May 31, 2023
Revision received: June 15, 2023
Accepted for publication: June 15, 2023




***Supplementary Fig 1.*** *Muscle-biopsy features of the described MDC1A newborn. (A-B) Frozen sections double immunolabeled with anti-laminin-α2 (merosin, green; Monosan, clone Mer3/22B2) and anti-pan-laminin (laminin-α2-β1-γ1, red; Sigma-Aldrich, code L9393) showing (A) a lack of basement membrane laminin-α2 staining in all myofibers and hyper-expression of -β1-γ1 subunits in MDC1A, but (B) colocalization of both markers in control muscle (CM). (C) Single immunolabeling confirms the absence of laminin-α2. (D) Compensatory expression of embryonic laminin-α1 (Novus Biologicals, code CL3183) and (E) laminin-α4 (Novus Biologicals, code NBP2-42393) in MDC1A compared to control muscle (insets). Scale bars: A-E: 50 μm.*




***Supplementary Fig 2.*** *Western blotting analysis of p62 and LC3 in the MDC1A newborn, the NM newborn, the anti HMGCR-positive IMNM patient, a DMD patient and an asymptomatic subject with elevated serum creatine kinase (CTRL).*




***Supplementary Fig 3.*** *Predominant fast myofibers in the MDC1A newborn. (A) An altered checkerboard pattern with predominant darkly stained type 2 myofibers is visible at histoenzymatic stain ATPase 9.4. (B) The size and proportion of different myofiber types are summarized in the histogram (continuous lines describe the MDC1A-affected newborn, whereas dotted lines are derived from published cases11 of healthy babies=HB) Scale bar: 50 µm.*