Supplementary eMethods Svahn et al :

Genetic analyses

Patients' DNAs were extracted from peripheral blood with Nucleospin Blood Kit (Macherey Nagel, Düren, Germany) and qualitatively checked using Nanodrop® One (Thermo Scientific, Wilmington, DE, USA). Custom targeted gene enrichment and DNA library preparation were performed using the Nimblegen EZ choice probes and Kapa HTP Library preparation kit according to the manufacturer's instructions (Nimblegen, Roche Diagnostics, Madison, Wisconsin). A specific custom panel of 40 genes was designed including genes associated with limb girdle muscular dystrophy (LGMD). The RefSeq coding sequences were determined as consensual for genetic diagnosis within a French nationwide working group. The targeted regions include all coding exons and ± 50 base pairs of flanking intronic regions of 40 genes known to be involved in LGMD. Paired-end sequencing was performed on a 300 cycles Flow Cell (Illumina, Santa Cruz, California) using the Illumina NextSeq platform. Twenty-four libraries were multiplexed per run. All non-polymorphic significant variants were confirmed by Sanger sequencing. Molecular study of CAVIN-1 gene used amplification of exons and flanking intronic regions by PCR followed by bi-directional capillary sequencing on ABI3500XL (AppliedBiosystems). Sequences produced were compared to the human reference genome (GRCh37/hg19) with the SeqScape v3 software (LifeTechnologies). Primers sequences are available on request.

Antibodies:

Primary antibodies: The anti-GAPDH (CST2118; WB 1:10,000) and the anti-Cavin-1 (D1P6W; WB 1:1000; IF 1:100) were from Cell Signaling Technology. The anti-Caveolin-3 antibody (610421; IHC 1:800) was from BD bioscan. The anti-myosin heavy chain (MHC) antibody

(MF20 hybridoma cell culture supernatant; WB 1:100) was from Developmental Studies Hybridoma Bank (DSHB). The anti-MURC/Cavin-4 (ab121642 or NBP1-86609, WB 1:2,000; IF 1:100; IHC 1:500) were from Abcam or Novus Biological respectively and correspond to the same antibody. The anti-Flag (F3165; WB 1:3,000), was from Sigma. The anti-MHC-I (M0736; IHC 1:50), the anti-C5b9 (DAKO, M0736; IHC 1:200) were from Dako and the anti-Dysferlin (NCL-Hamlet; IHC 1:800) was from Leica. The anti-CD56 (NCL-CD56-1B6) was from Novocastra. The HLA-DR (AB_2848953; IHC 1:100) was from Invitrogen.

Secondary antibodies: For Western blotting, the anti-mouse (NA931V) and anti-rabbit (NA934V) labeled with peroxidase (GE Healthcare UK) were used at 1:10,000. The anti-human total IgG (709-036-149; WB 1:10,000) from Jackson Immuno Research or anti-human IgG1 (MH1715), IgG2 (MH1722), IgG3 (MH1732), IgG4 (MH1742) from Invitrogen labeled with peroxidase were used at 1:1,000.

For immunofluorescence, the goat anti-human-Alexa568 (A21090) and the donkey anti-rabbit alexa-Fluor 488 (A21206) were purchased from Molecular probe. All antibodies were used at 1:1,000.

Patient muscle biopsy and immunohistochemistry studies:

Fresh deltoid muscle specimens were divided into two fragments, one was fixed in 10% neutral buffered formalin and embedded in paraffin (Formalin-Fixed Paraffin-Embedded - FFPE sections), the other frozen in isopentan. Three µm-thick longitudinal and transversal sections were cut either with a microtome or a cryostat for morphological and immunohistochemistry (IHC) stainings. Hematoxylin-phloxine-saffron (HPS) staining was used for morphological analysis both in fixed and frozen sections. An automated IHC analysis of the frozen sections was performed on a BenchMark XT (Ventana Medical Systems Inc., Tucson, AZ, USA) using anti-MHC-I, anti-Caveolin-3, anti-Dysferlin or anti-MURC/Cavin-4 antibodies at established

final concentrations according to the manufacturer's instructions and revealed by avidin-biotin-peroxidase complex and Ventana DAB detection and amplification kits. Sections were counterstained with hematoxylin. Slides were scanned using a Zeiss Axio Observer Z1 microscope.

Frozen Non-iRMD B2-thymoma and iRMD B2-thymoma were obtained from Dr Lara Chalabreysse.

All Human biological samples and associated data were obtained from Tissu-Tumorothèque Est and CBC Biotec Biobank (CRB-HCL Hospices Civils de Lyon NF S 96900 certification BB-0033-0046) a center for biological resources authorized by the French Ministry of Research (AC-2019-3465).

Electron microscopy studies:

For ultrastructural electron microscopy studies, biopsy samples were fixed with 2% glutaraldehyde (Electron Microscopy Sciences - EMS) in 0.1 M sodium cacodylate (pH 7.4) buffer. After washing three times in 0.2 M sodium cacodylate buffer, tissues were post-fixed with 2% osmium tetroxide (EMS) at room temperature for 1 hr and dehydrated in a graded series of ethanol at room temperature, transferred to propylene oxide (EMS) and embedded in Epon epoxy resin (EMS). After polymerization, ultrathin sections (100 nm) were cut on a UC7 ultramicrotome (Leica) and collected on 200 mesh grids. Sections were stained with uranyl acetate and lead citrate before observations on a Jeol 1400JEM (Tokyo, Japan) transmission electron microscope equipped with a Orius 600 camera and Digital Micrograph.

Cells:

The human Y711i myoblast and the HEK293T cell lines were previously described in Coudert *et al.,* (2021) [1]. The Y711i Myoblast (MB) cells were cultured in the KMEM proliferation

medium (1 volume of M199, 4 volumes of Dulbecco's modified Eagle's medium DMEM-Glutamax-4,5g/L glucose with pyruvate, 20% fetal bovine serum (v/v), 25 μg/mL Fetuin, 0.5 ng/mL β-FGF, 5 ng/mL EGF, 5 μg/mL Insulin, dexamethasone 0.2 μg/mL and penicillin streptomycin P/S). Differentiation of Y711i MB into Y711i myotubes (MT) was carried out in differentiation medium (DMEM-glutamax, 4,5g/L glucose and pyruvate; M199 medium; 1% horse serum, insulin 10 μg/mL) for 4-5 days. The human embryonic kidney cell line (HEK293T) was obtained from Généthon (Evry, France) and were cultured in DMEM (Glutamax, 4,5 g/L glucose and pyruvate) supplemented with 10% (v/v) FBS and P/S. All components and media were provided by Gibco.

Plasmid Constructs:

The pcDNA3.1^{+}-C-Flag-CAVIN1 (OHu23701D), CAVIN2 (OHu55607D), CAVIN3 (OHu31085D) and CAVIN4 (OHu20583D) were provided by GenScript.

Transfection

HEK293T cells were transfected in 6 well plates (4,5-5 x 10^5 cells/well plated 1 day before) with 2 μg of the different pcDNA3.1^{+}-C-Flag-CAVIN1-4 constructs using the Fugene HD (Promega) according to the protocol provided by the manufacturer.

Western blotting

Human Y711i myoblasts and myotubes were harvested in cold PBS and lyzed in loading buffer (60 mM Tris HCl buffer at pH 6.8, 2% SDS, 10% Glycerol, 5% β-mercapto-ethanol, 0.025 % bromophenol blue) containing anti-proteases (Complete EDTA-free, Sigma) and sonicated. Protein concentration was determined with the Bradford reagent (Bio-Rad Laboratories) and 5-10μg of proteins were loaded and separated by SDS-PAGE electrophoresis gels in presence of

2,2,2-Trichloroethanol (TCE, T54801, Sigma Aldrich) for stain free gel analyzes. Gels were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, IPVH0001 0.45 µm, Millipore) by semi-dry electrotransfer apparatus (TransBlot® TurboTM, Bio-Rad Laboratories) in TOWBIN buffer (25 mM Tris, 192 mM glycine, 20% methanol). Membranes were incubated with primary antibody in 1% milk in Tris-Buffered Saline (TBS buffer; UP74004B, Interchim) containing 0.1% Tween-20 (TBST buffer) over-night at 4°C. Pre and post treatment patient serum (PS and TPS respectively) as well as healthy control serum HCS were used at 1:10,000 in presence of milk. Membranes were successively washed 3x5min in TBST and were next probed with horseradish peroxidase-conjugated secondary antibodies for 1 hr at room temperature. After three washing steps, immunodetection was carried out using the ECL™ reagent (Amersham). WB revelation was carried with a ChemiDocTM Touch imaging system and quantification of immunoblots was done using the image lab software (Bio-Rad Laboratories).

For preparation of total lysates from non-iRMD and iRMD B2-thymoma biopsies, samples were lyzed in HS buffer (10mM Tris HCl pH7.5;150mM NaCl;0.5mM EGTA; 1mM DTT; anti-proteases) in 2 ml-tube from the lysing kit, containing ceramic beads using the precellys homogenizer device (Bertin Instruments). Lysates were solubilized in loading buffer and sonicated. Samples were analyzed by Western blotting as previously described above.

Immunofluorescence and confocal microscopy imaging

Differentiation of myoblasts into myotubes was carried out on 12-mm diameter coverslips in 6 well plates, 35-mm dishes or in 4 wells-labtek (Thermo Fischer Scientific) and fixed with 4% paraformaldehyde (PFA) in 1X-PBS at room temperature for 10 min. Cells were permeabilized with 0.2% Triton X-100 (Sigma Aldrich) for 5 min at room temperature and blocked 1 hr with 1X-PBS containing 2% BSA. Myotubes were labeled with the indicated primary antibody in

1X-PBS containing 2% of BSA overnight at 4°C. PS, TPS and HCS sera were used at 1:500 dilution. After extensive washing steps in PBS, myotubes were stained for 1 hr at room temperature with secondary antibodies coupled with alexa fluor 488 or 568. After washings, nuclear DNA was counterstained with DAPI (D9542; Sigma-Aldrich) for 5 min at room temperature and washed 3 times. Images were acquired using the confocal microscope Zeiss LSM-880 (CIQLE platform from Faculty of Medicine of Lyon, France) and analyzed using the Image J software (version 1.8).

Immunoprecipitation and mass spectrometry based proteomic assay:

Myotubes were lyzed in RIPA buffer (R0278 Sigma) containing anti-proteases (cOmplete, Mini, EDTA-free protease Inhibitor Cocktail, Sigma). Cell lysate was sonicated and 870 µg of total protein were used for immunoprecipitation using Dynabeads™ Protein G magnetic beads (Invitrogen) coated with control (HCS) or rippling patient (PS) sera overnight at 4°C. After extensive washing steps, control and rippling beads were resuspended in 2X Loading buffer, heated at 96°C during 5 min and loaded on precast SDS-PAGE 10% (Biorad). One part of the immunoprecipitates was used for WB using PS (1:10,000) as quality control and the second part was used for the mass spectrometry analyses. The analytical gel was Coomassie blue stained and bands corresponding to the 50-55-kDa recognized antigen and comigrating with IgG heavy chains were cut out and sent to mass spectrometry/MS-based proteomic analyses.

The proteins were digested in-gel using modified trypsin (sequencing purity, Promega), as previously described [2]. The resulting peptides were analyzed by online nanoliquid chromatography coupled to MS/MS (Ultimate 3000 RSLCnano and Q-Exactive Plus, Thermo Fisher Scientific) using a 80 min gradient. For this purpose, the peptides were sampled on a precolumn (300 µm x 5 mm PepMap C18, Thermo Scientific) and separated in a 75 µm x 250 mm C18 column (Reprosil-Pur 120 C18-AQ, 1.9 µm, Dr. Maisch). The MS and MS/MS data

were acquired by Xcalibur (Thermo Fisher Scientific). Peptides and proteins were identified by Mascot (version 2.8.0, Matrix Science) through concomitant searches against the Uniprot database (*Homo sapiens* taxonomy, 20220527 download) and a homemade database containing the sequences of classical contaminant proteins found in proteomic analyses. Trypsin/P was chosen as the enzyme and two missed cleavages were allowed. Precursor and fragment mass error tolerances were set at respectively at 10 and 20 ppm. Peptide modifications allowed during the search were: Carbamidomethyl (C, fixed), Acetyl (Protein N-term, variable) and Oxidation (M, variable). The Proline software [3] was used for the compilation, grouping, and filtering of the results (conservation of rank 1 peptides, peptide length ≥ 6 amino acids, false discovery rate of peptide-spectrum-match identifications < 1% [4], and minimum of one specific peptide per identified protein group). Proline was then used to perform a compilation, grouping and spectral counts-based comparison of the protein groups identified in the different samples. To be considered as a potential target, a protein must be identified only with SP with a minimum of 5 specific spectral counts or enriched at least 10 times with SP compared to HSC.

Supplementary references associated with eMethods

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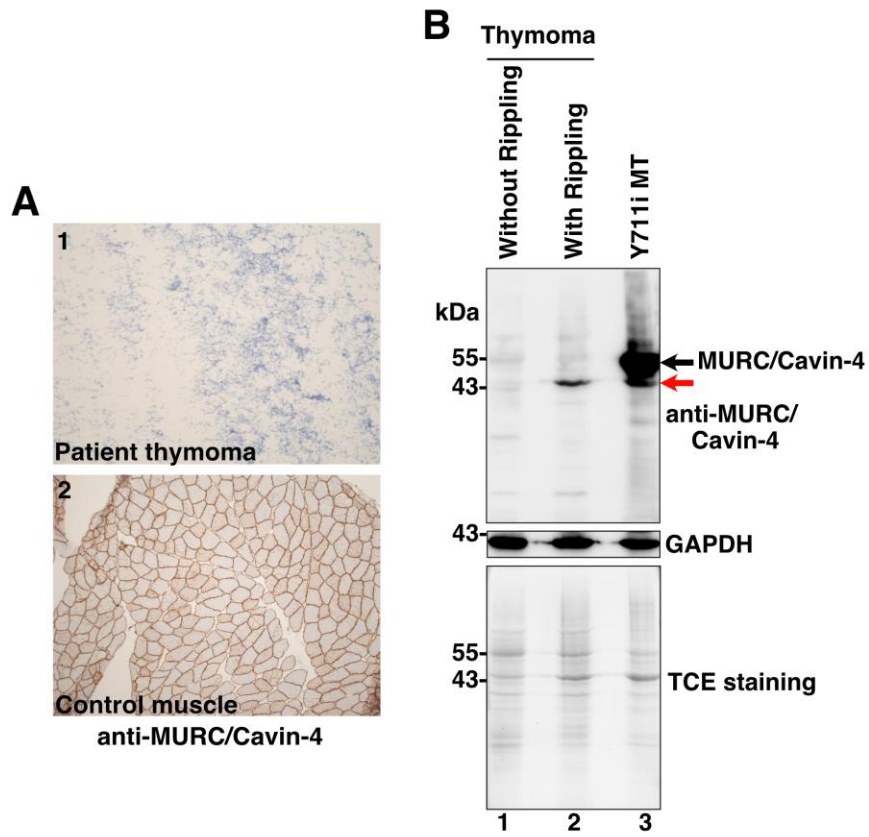
2. Casabona MG, Vandenbrouck Y, Attree I, Couté Y. Proteomic characterization of Pseudomonas aeruginosa PAO1 inner membrane. Proteomics. 2013;13(16):2419-2423. doi:10.1002/pmic.201200565

3. Bouyssié D, Hesse AM, Mouton-Barbosa E, et al. Proline: an efficient and user-friendly software suite for large-scale proteomics. Valencia A, ed. Bioinformatics. 2020;36(10):3148-3155. doi:10.1093/bioinformatics/btaa118

4. Couté Y, Bruley C, Burger T. Beyond Target–Decoy Competition: Stable Validation of Peptide and Protein Identifications in Mass Spectrometry-Based Discovery Proteomics. Anal Chem. 2020;92(22):14898-14906. doi:10.1021/acs.analchem.0c00328



Supplementary-FIGURE 1 Svahn et al.,

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3 **eFigure 1:** MURC/Cavin-4 expression in B2-thymoma biopsies.

4 (**A**) IHC analysis of MURC/Cavin-4 in the B2-thymoma Rippling patient section (**A1**) and on
5 a muscle section of an healthy control individual (**A2**). Magnification x100. (**B**) Western
6 blotting analysis of MURC/Cavin-4 expression on non-iRMD B2-thymoma (lane 1), iRMD
7 B2-thymoma patient (lane 2) and Y711i myotubes MT (lane 3) using the anti-MURC/Cavin-4
8 commercial antibody or the anti-GAPDH as a loading control. TCE staining was used as
9 loading and protein distribution controls. Note the absence of the 55kDa MURC/Cavin-4
10 typical band in both thymomas compared to the MT extract positive control. Note the presence
11 of a MURC/Cavin-4 immunoreactive 43 kDa band in iRMD B2-thymoma and Y711i MT (red
12 arrow).

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