

## Supplementary methods

**Generation of tg(HSA:LT $\alpha$ \_HSA:LT $\beta$ ) mice.** Lymphotoxins were amplified from plasmid DNA by the following primers: LT $\alpha$  fw BamHI (5'CGG GGATCC CAG ACT ATC ATC ATG ACA CTG CTC GGC CGT CTC C3'), LT $\beta$  fw BamHI (5'CGG GGATCC CAG ACT ATC ATC ATG GGG ACA CGG GGA CTG CAG G3'), LT $\alpha$  rev AscI-EcoRI (5'CCGG GAATTC GGCGCGCC TTC CTA CAG TGC AAA GGC TCC3') and LT $\beta$  rev AscI-EcoRI (5'CCGG GAATTC GGCGCGCC CCA TCA CCC CAC CAT CAC CGC3') respectively. A plasmid containing the human alpha-skeletal actin (HSA) promoter (-1905 to +239) was kindly provided by Edna Hardeman.<sup>1</sup> After digest with BamHI and EcoRI, lymphotoxins were cloned downstream of the HSA promoter, a SV40 poly A tail was added by directional cloning using AscI and NotI. After linearization, HSA-LT $\alpha$  and HSA-LT $\beta$  transgenes were used for pro-nuclear co-injection and embryos were then transferred into pseudo-pregnant C57BL/6J at Biomodels Austria GmbH (Biat) by Prof. Dr. Thomas Rüllicke. We obtained four founders that carried both transgenes HSA-LT $\alpha$  and HSA-LT $\beta$ , all four transmitted the transgenes through the germline. Animals were maintained on a C57BL/6J background under SPF conditions.

**Genotyping.** DNA was extracted from tail or ear biopsies. Transgene-positive tg(HSA:LT $\alpha$ \_HSA:LT $\beta$ ) mice were identified by PCR using the following primers: HSA fw: CAG TCG GTT CAC CTG GTC AG combined with LT $\alpha$  rev: ACC CTC AAG AGG TGG AGA CG or LT $\beta$  rev: AGG CCA GCA CAG CCA GGA CA. Atg5<sup>fl/fl</sup> were genotyped as follows. We used a combination of primers: primer „exon3-1“: GAA TAT GAA GGC ACA CCC CTG AAA TG, Primer „short2“: GTA CTG CAT AAT GGT TTA ACT CTT GC, Primer 3 „check2“: ACA ACG TCG AGC ACA GCT GCG CAA GG to distinguish the wildtype (exon3-1 and short2) from the floxed Atg5 allele (check2 and short2). To detect the presence of Cre in Ckmm-Cre mice, we used the primers: Cre fw: AGC GAT GGA TTT CCG TCT CT and Cre rev: CAC CAG CTT GCA TGA TCT CC.

**Quantitative real time PCR.** We extracted mRNA from various tissues by mechanical homogenization in Trizol followed by purification using RNeasy Kit (Qiagen) or RNeasy Plus Universal Mini Kit (Qiagen). We then reversely transcribed the RNA into cDNA with the Superscript Kit. Quantitative real time PCR was performed using Sybr Green and the

primers listed in Supplementary Table 1. QPCR results were displayed graphically by heat maps that we created using the ComplexHeatmap package in Bioconductor/ RStudio Version 4.3.0.<sup>2,3</sup>. Each sample was visualized by an individual row in the heat map, hence the number of samples (different mice) for each experiment can be seen in the corresponding figures. We only excluded individual samples that retrospectively turned out to be of the wrong genotype. Experiments were not blinded. No a priori sample size calculation was done.

The following quantitative PCR (qPCR) primers were used:

<b>mouse gene</b>	<b>sequence of forward primer 5'→3'</b>	<b>sequence of reverse primer 5'→3'</b>
A20	TGGTTCCAATTTTGCTCCTT	CGTTGATCAGAGTCGTG
Atf3	GAGGATTTTGCTAACCTGACACC	TTGACGGTAACTGACTCCAGC
Atf4	ATGATGGCTTGCCAGTG	CCATTTTCTCCAACATCCAATC
Atg3	GGGAAGCTGCAGACATGGAA	GCCAAACAACCATAGCCGTG
Atg5	TGTGCTTCGAGATGTGTGGTT	GTCAAATAGCTGACTCTTGCCAA
Atg12	AAGAAATGGGCTGTGGAGCG	TGCAGTAATGCAGGACCAGT
App	AGCCCAGAATCAGCTACGGAA	GCTCCACGGTGGTCTTGTT
B2m	CTCGGTGACCCTGGTCTTTC	GGATTTCATGTGAGGCGGG
Baff	CAGCGACACGCCGACTATAC	TAGCAAAGATGGGGTCCGTG
Beclin-1	GGAAGTAGCTGAAGACCGGG	TTAGACCCCTCCATGCCTCA
Cathepsin B	GTACTIONAGGAGTGACGGGAG	TGTAGAAGTTGCGTCCAGCC
Ccl2	TTAAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Ccl5 (RANTES)	AGATCTCTGCAGCTGCCCTCA	GGAGCACTTGCTGCTGGTGTAG
Ccl17	TACCATGAGGTCACTTCAGATGC	GCA CTC TCG GCC TAC ATT GG
Ccl19	GCCTCAGATTATCTGCCAT	AGACACAGGGCTCCTTCTGGT
Ccl20 (1)	ACTGTTGCCTCTCGTACATACA	ACCCACAATAGCTCTGGAAGG
Ccl20 (2)	TTTTGGGATGGAATTGGACAC	TGCAGGTGAAGCCTTCAACC
Chop	GCGACAGAGCCAGAATAACA	GATGCACTTCCTTCTGGAACA
Cox4i1	ATTGGCAAGAGAGCCATTTCTAC	CACGCCGATCAGCGTAAGT
Cox4i2	CTGCCCGGAGTCTGGTAATG	GTAGCAGTCAACGTAGGGGG
Cox5a	TGATGCCTGGGAATTGCGTA	AGATGCGAACAGCACTAGCA
Cpt1a	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
Cryab	CGGACTCTCAGAGATGCGTT	TGGGATCCGGTACTTCCTGT
Cxcl9	CTTCGAGGAACCCTAGTGATAAGG	CCTCGGCTGGTGTCTGATG
Cxcl10	AAGTGCTGCCGTCATTTTCT	CCTATGGCCCTCATTCTCAC
Cxcl13	ATATGTGTGAATCCTCGTGCCA	GGGAGTTGAAGACAGACTTTTGC
Cycs	GAGGCAAGCATAAGACTGGACC	ACTCCATCAGGGTATCCTCTCC
Ero1a/ Ero1l	GCGTCCAGATTTTCAGCTCT	TCGAAGTGCAAAGGAAATGA
Gadd34	GAGGGACGCCCAACTTC	TTACCAGAGACAGGGGTAGGT
Gapdh	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT
Gpx1	AGTCCACCGTGTATGCCTTCT	GAGACGCGACATTCTCAATGA
Gpx2	GCCTCAAGTATGTCCGACCTG	GGAGAACGGGTCATCATAAGGG
Gpx3	CCTTTTAAGCAGTATGCAGGCA	CAAGCCAAATGGCCCAAGTT
Gpx4	TTTACGAATCCTGGCCTTCCC	CACGCAGCCGTTCTTATCAA
Gpx7	TCCGAGCAGGACTTCTACGAC	TCTCCCTGTTGGTGTCTGGTT
Gpx8	ACGGAGTCACATTCCCCATC	CTGGCCTCCAGAACTTCACG

Grp78	CTGAGGCGTATTTGGGAAAG	TCATGACATTCAGTCCAGCAA
Hspa9	ATGGCTGGAATGGCCTTAGC	ACCCAAATCAATACCAACCACTG
Hspe1	AGTTTCTTCCGCTCTTTGACAG	TGCCACCTTTGGTTACAGTTTC
Hsp27	GGGCAGTACTTGGGATCAGG	GCCGAACATAGTAGCCGTGA
Hsp60	TCTTCAGGTTGTGGCAGTCA	CCCCTCTTCTCCAAACACTG
Hsp90a	CTCGTGCGTGTTCA TTCAGC	CCAGTTTACTGGGGTCCGTC
Hsp90b	ACTACTACTCGGCTTTCCCGT	TAGAGATCAACTCGCGGAGGA
Ifng	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCATG
Il1b	TCCCGTGGACCTTCCAGGATGA	GGGAACGTCACACACCAGCAGG
Il6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
Light	TCCGCGTGCCTGAAAA	AAGCTCCGAAATAGGACCTGG
Lta	TCCACTCCCTCAGAAGCACT	AGAGAAGCCATGTCCGAGAA
Ltb	TACACCAGATCCAGGGGTTTC	ACTCATCCAAGCGCCTATGA
Ltbr	GCCGAGGTCACAGATGAAAT	CAGGACACTGGTGAAGAGCA
Mhcl	TACCTGAAGAACGGGAACGC	CAAGAGGCACCACCACAGAT
mt-Cox1	AGCCTGAGCGGGAATAGTG	ATGGGCAGTTACGATAACATTGT
mt-Tfa	AGCGTGCTAAAAGCACTGGG	CTCCACAGGGCTGCAATTTT
Murf1	AGCATCAAGATCCGTCTGACA	CCAGAGCCGTCCACAACAAT
Myod	ATCCGCTACATCGAAGGTCTG	GGTGTCTAGCCATTCTGCC
Ncf2 (1)	GCTGCGTGAACACTATCCTGG	AGGTCGTA CTCTCCATTCTGTA
Ncf2 (2)	CTAAACTGAGCTACCGGCGT	ACCGTATGCTCACACCACAG
Nqo1	TTTAGGGTCGTCTTGGAAC	GTCTTCTCTGAATGGGCCAG
Nrf1	CTTCATGGAGGAGCACGGAG	CGGCCATTCCACAGGAC
Nrf2	TAGATGACCATGAGTCGCTTGC	GCCAACTTGCTCCATGTCC
p58ipk/Dnajc3	GGCGCTGAGTGTGGAGTAAAT	GCGTGAAACTGTGATAAGGCG
Pax7	GCTACCAGTACAGCCAGTATG	GTCATAAGCATGGGTAGATG
Pgp1a	TTGACTGGCGTCATTCCGGG	TCGCAGGCTCATTGTTGTACT
Pgp1b	ATCTCTCTGACACGCAGGGT	CTTCGTAAGCGCAGCCAAGA
Ppargc1a	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
Pprc1	TCTGTGAGTCTGTTGGAGC	AATGCCTGAGTTTCCTACAGC
Rpl32	GGGAGCAACAAGAAAACCAA	ATTGTGGACCAGGAAC TTGC
Slc41a3	CTCAGCCTTGAGTTCCGCTTT	GCAGGATAGGTATGGCGACC
Sod1	AACCAGTTGTGTTGTCAGGAC	CCACCATGTTTCTTAGAGTGAGG
Sod2	CAGACCTGCCTTACGACTATGG	CTCGGTGGCGTTGAGATTGTT
Sod3	CCTTCTTGTTCTACGGCTTGC	TCGCCTATCTTCTCAACCAGG
s-Xbp1	TGACGAGGTTCCAGAGGTG	TGCACCTGCTGCGGACTCAG
Tfam	ATTCCGAAGTGTTTTTCAGCA	TCTGAAAGTTTTGCATCTGGGT
Tgfb1	ATCCTGTCCAAACTAAGGCTCG	ACCTCTTTAGCATAGTAGTCCGC
Tid1/Dnaja3	GCTCGGGCATGGAACTATCA	TCTGCACCCTGAATGTGACAA
Tnfa	CGATGGGTTGTACCTTGTC	CGGACTCCGCAAAGTCTAAG
Tnfr1	ACCAAGTGCCACAAAGGAAC	CACGCACTGGAAGTGTGTC
Tnfr2	GACTGTGAGGCAAGCATGTAT	GGTTCTGCTGTTTAGTGCAGG
Trail	ATGGTGATTTGCATAGTGCTCC	GCAAGCAGGGTCTGTTCAAGA
Trib3	GCAAAGCGGCTGATGTCTG	AGAGTCGTGGAATGGGTATCTG
Tweak	CCGAGCTATTGCAGCCCATT	CAGACACCTGGCACAAACGG
u-Xbp1	GCAGCACTCAGACTATGT	GGTCCAACTTGTCAGAAATGCCC

Human primers	Sequence	
ATG5	AAAGATGTGCTTCGAGATGTGT	CACTTTGTCAGTTACCAACGTCA
BAFF	CTCAAGACTGCTTGCAACTGA	AGCTGAGAAGCCATGGAACA
BECN1	CCATGCAGGTGAGCTTCGT	GAATCTGCGAGAGACACCATC
CCL2	CTTCGGAGTTTGGGTTTGCTT	CATTGTGGCCAAGGAGATCTG
CCL4	CAATACCATGAAGCTCTGC	GGAGTCCTGAGTATGGAGG
CCL5	CCCCTCACTATCCTACC	TCACGCCATTCTCCTG
CCL17	ACCGTTGGTGTTACCCGCCC	GGCCCTTTGTGCCCATGGCT
CXCL10	TATTCCTGCAAGCCAATTTTGT	TCTTGATGGCCTTCGATTCTG
CCL19	CTGCTGGTTCTCTGGACTTCC	AGGGATGGGTTTCTGGGTCA
CCL20	GCTACTCCACCTCTGCGGCG	CAGCTGCCGTGTGAAGCCCA
CCL21	CAAGCTTAGGCTGCTCCATC	TCAGTCCTCTGCAGCCTTT
CTSB	AGAGTTATGTTTACCGAGGACCT	GATGCAGATCCGGTCAGAGA
HPRT1	TGACACTGGCAAACAATGCA	GGTCCTTTTCACCAGCAAGCT
IFNg	CCAACGCAAAGCAATACATGA	CCTTTTTCGCTTCCCTGTTTTA
IL6	TCGAGCCCACCGGGAACGAA	GCAACTGGACCGAAGGCGCT
LIGHT	CTGGCGTCTAGGAGAGATGG	CTGGGTTGACCTCGTGAGAC
IL1b	ATGATGGCTTATTACAGTGGA	GTCGGAGATTCTGAGCTGGA
LTa	GCTCACCTCATTGGAGACCC	ACCACCTGGGAGTAGACGAA
LTb	GGACTGGTAACGGAGACGG	CTTCAGCGGAGCGCCTAT
TGFb	CTAATGGTGGAACCCACAACG	TATCGCCAGGAATTGTTGCTG
TNFa	GGCGCTCCCCAAGAAGACAGG	CCAGGCACTCACCTCTTCCCT
TRAIL	TGCGTGCTGATCGTGATCTTC	GGGGTCCCAATAACTGTCATCTT
TWEAK	CGCCTTTCCTGAACCGACTA	CCGTGTTTTCCGGCCTTTA

## Histology, immunohistochemistry, muscle fiber size determination.

Tissues were fixed in 4% neutral buffered formalin for a minimum of 24 h and then paraffin-embedded. Experiments were not blinded. 2  $\mu$ m sections were cut from paraffin blocks with a microtome, mounted on glass coverslips (Thermofisher) or X-tra adhesive white slides (Leica, #3800200AE), and stained using a Leica BOND-MAX automated staining machine unless otherwise noted. Stains were developed with 3,3'-diaminobenzidine (DAB) and/or Fast Red (Sigma), counter-stained with hematoxylin and eosin (H&E), cleared with xylol, mounted, and imaged with a Zeiss Axioscope A1 light microscope. Alternatively, muscle was glued to cork using tragacanth gum and snap frozen in an isopentane bath cooled by liquid nitrogen. 2  $\mu$ m cryosection were made using a cryo microtome. Muscle fiber size distribution was determined on H&E stained cross sections of the quadriceps femoris muscle, using ImageJ. The largest diameters of at least 100 cross-sectioned muscle fibers per animal were measured and displayed in histograms. Gomori trichrome staining and MHCI immunohistochemistry were also performed on cryotissue sections. Histological analysis was not blinded. We are not aware of

any potential confounders and therefore did not control for them. A sample size of at least 3 per condition was deemed appropriate for analysis. No a priori sample size calculation was done.

MHCI immunolabeling on cryosections of mouse muscle was performed using rat anti-MHCI antibody at 1:500 dilution (Abcam, ab15681) using the Bond Polymer Refine Red Detection kit (Leica, DS9390). At least n=3 samples (different mice) were stained and analyzed per genotype and timepoint.

Mouse paraffin sections were immunostained on the Leica BOND-MAX with rabbit anti-Rel B (C19) antibody (Santa Cruz, sc-226) at 1:400 dilution (pretreatment: EDTA for 20 min. at 100° C); rabbit anti-Rel A antibody (Novus Biologicals, NB100-2176) at a 1:500 dilution (pretreatment: EDTA for 30 min. at 100° C); rabbit anti-CD68 antibody (Abcam, ab125212) at a 1:100 dilution (pretreatment: citrate buffer for 30 min. at 100° C); rat anti-CD4 (Thermofisher, 14-9766-82) at 1:1000 dilution (pretreatment: EDTA for 30 min. at 100° C); rabbit anti-CD3 (Invitrogen, MA1-90582) at 1:500 dilution (pretreatment: EDTA for 30 min. at 95° C); rat anti-LAMP2 (Santa Cruz, sc19991) at 1:100 dilution (pretreatment: EDTA for 20 min. at 100° C); rat anti-CD45R/B220 (BD, 553084) at 1:3000 dilution (pretreatment: EDTA for 20 min. at 100° C); rat anti-CD8 alpha (Invitrogen, 14-0808-82) at 1:200 dilution (pretreatment: EDTA for 20 min. at 100° C; primary antibody incubation for 1 h). Secondary antibodies: rabbit anti-rat (Jackson Immuno Research, 312-005-045) at 1:1000 dilution, in 2% mouse serum; anti-rabbit HRP antibody was part of the Bond Polymer Refine Detection Kit (Leica, DS9800). Unless otherwise stated, primary antibodies were incubated for 30 min at room temperature, secondary antibodies for 20 min at room temperature. At least n=3 samples (different mice) were stained and analyzed per genotype and timepoint for each staining.

P62 and pTDP43 staining were performed manually: 5-6 µm paraffin sections were placed on poly-L-lysine coated slides and allowed to dry in an oven (37° C) overnight and then processed for immunohistochemistry described.<sup>4,5</sup> Sections deparaffinized in xylene for 20 min were rehydrated in 100%, 95% and 70% ethanol for 5 min each followed by endogenous peroxidase quenching (0.3% H<sub>2</sub>O<sub>2</sub> in methanol) for 20 min. For antigen retrieval, sections were heated in citrate buffer, pH 6 (DAKO), for 20 min in a pressure cooker. After washing in PBS, sections were incubated with primary antibody (anti-pTDP43, mouse TIP-PTD-M01, Cosmo Bio Co.LTD or P62/SQSTM1 rabbit P0067, Sigma Aldrich) for 1 h at room temperature or at 4° C overnight. After washing in PBS, sections were incubated with appropriate polymeric HRP-linker secondary antibody (IL Immunologic, Duiven, Netherlands) for 30 min at room temperature. Diaminobenzidine (DAB) reagent (DCS Innovative Diagnostic System DAB kit)

was used to stain the sections which were then counter-stained with 6% hematoxylin for 3 min. All procedures were performed at room temperature.  $N=6$  samples (different mice) were stained and analyzed per genotype.

Ubiquitin immunostaining was done with the Ventana BenchmarkGX Ultra View DAB Detection kit. Deparaffinization and pretreatment was done with protease 1 for 16 min. Polyclonal rabbit anti-Ubiquitin antibody (Dako, Z0458) was incubated for 32 min at a 1:100 dilution. Sections were then counter-stained with hematoxylin for 8 min and with blueink reagent for 4 min.  $N=4$  samples (different mice) were staining and analyzed per genotype, except for HSA-LT;CreAtg5, we stained and imaged  $n=3$  samples (mice).

Combined COX-SDH enzyme histochemistry was performed on 7 $\mu$ m cryo sections. Slides were incubated for 1 h at RT in COX incubation solution (50mM phosphate buffer, pH 7.4, 243mM sucrose [Sigma S5391], 90mM cytochrome C [Sigma C7752] and containing 0.22mg/ml catalase [Sigma C40] as well as 3,3'-diaminobenzidine tetrahydrochloride [DAB; Sigma D5905], followed by a 2min wash in aqua dest and then exposed to SDH incubation solution (200mM phosphate buffer, 200mM sodium succinate dibasic hexahydrate [Sigma S2378], containing 1mg/ml Nitro-Blue-Tetrazolium [NBT; Serva 30550], pH 7.4) for another 1 h at 37°C. After a final wash step in aqua dest, slides were mounted in glycerine-gelatine.

**Electron microscopy.** Samples were fixed in 3% glutaraldehyde in Sørensen buffer, incubated in 1% osmium, then dehydrated through a series of increasing concentrations of ethanol and finally placed in 100% propylenoxide. Samples were then embedded in epon resin, containing glycidether 100, 2-dodecenylsuccinic acid anhydride, Renlam M-1, phthalic acid-di-butylester and 2,4,6-Tris(dimethylaminomethyl)phenol (all from Serva). Resin was hardened over night at 75°C. Ultrathin resin sections were examined by electron microscopy (EM) using a CM100 Philips or a Hitachi HT7700 transmission electron microscope (TEM).  $N=3$  animals HSA-LT;CreAtg5, 6 months of age were analyzed and compared to  $n=4$  wild-type and  $n=2$  HSA-LT $\alpha/\beta$  mice, both at 6 months of age. For the analysis of mitochondrial morphology, we examined mice around 9 months of age:  $n=5$  HSA-LT $\alpha/\beta$  compared to  $n=3$  wild-type mice.

**Forelimb grip strength test.** Forelimb grip strength was measured using a Grip Strength Meter (GPM-100; Melquest, Toyama, Japan). The peak pull force in grams was measured on a digital force sensor as the mouse grabbed the bar mounted on the force gauge. After stabilization, the gauge was reset to 0 g, and the mouse's tail was progressively pulled back by an investigator. The gauge registered the tension when the mouse removed its forepaws

from the bar. Excluded from each test were trials in which the mouse utilized just one forepaw or the hindlimbs and twisted during the pull or left the bar without resistance. Given that the speed of the tail pull might affect the measurement, we performed the technique at a constant pace slow enough to allow mice to develop resistance to it. We took 5 measurements in a row at one-minute intervals, each session's average performance is shown. The investigator was blinded to the mice's genotype. All test sessions were conducted during the light cycle's afternoon hours. The manufacturer calibrated the equipment on a regular basis.

**Hanging wire test.** The four-limb hang test employs a wire grid system to non-invasively assess the capacity of mice to maintain continuous limb tension in opposition to gravitational force. The process evaluates the 4 limb longest hanging time in seconds. The test can be used to identify the course of neuromuscular illness as well as the success of genetic or pharmacologic treatments. The animal is suspended from an elevated wire cage top at the start of the test. The animal is put on the top of the cage, which is then inverted and suspended above the home cage; the time between when the animal falls is recorded. This exam is run with three trials per session and a 5 min interval between trials. Each session's average performance is shown as the average of the three trials. All other conditions are as described above.

**Behavioral tests before and during drug treatments.** Behavioral tests before and during the drug treatments were performed as follows: Starting from 90 days of age, the mice had free access to a fully computerized running wheel (circumference 38 cm), which was equipped with an aligned rotation sensor (resolution of a one-sixteenth turn). The MATLAB software (MathWorks, Natick, MA, USA) recorded continuously the daily number of runs, maximum velocity, and cumulative running time and distance as described.<sup>6</sup>

**Muscle volumetry by MRI.** Spontaneously breathing mice were placed under isoflurane anesthesia on an MRI bed on a warming pad in supine position. Images were acquired using a 4.7 T Bruker's Pharma scan. A whole-body radio frequency transmitter coil and a QuadResonator receiver coil were used. The Bruker method nvsFLASH (fast low-angle shot) was run with the Bruker ParaVision 5.0 operating system and based on the Bruker gradient-echo sequence FLASH for the acquisition of multislice images. Data visualization and quantification were performed using Matlab.

**In situ RNA-hybridization.** RNA in situ hybridization was performed using the RNAscope 2.0 brown FFPE Assay for murine and the BOND RNAscope Brown Detection kit

(Advanced Cell Diagnostic/ Leica) for human tissue according to the manufacturer's protocol. Details are provided in the supplementary methods.

One tissue section per genotype and timepoint was used. In brief, 2µm (mouse) or 5 µm (human) paraffin embedded tissue sections on Fisherbrand Superfrost Plus™ Microscope slides were baked for 1h in a dry oven at 60°C. Deparaffinization and blocking of endogenous peroxidases was performed according to the assay protocol. For human tissue, an automated protocol was used on a BOND-III, Leica following the manufacturer's protocol (Bond RNAscope Brown Detection Kit DS9815). For murine tissue, the following steps were done manually: slides were cooked for 15 min in pretreatment solution 2 and protease digestion was performed for 30 min using pretreatment 3 solution. Murine LTα and LTβ or human LTα and LTβ R-specific probes (Advanced Cell Diagnostic/ Leica) were incubated for 2 h at 40°C (mouse) or 42°C (human). Signal amplification and detection were performed according to the assay protocol. Counterstaining with Gill's Hematoxylin I (American Master Tech Scientific) was performed as described in assay protocol. Slides were mounted using EcoMount mounting medium (BioCare).

## **Enzyme-linked immunosorbent assays (ELISA) for**

**LTα3/LTα1β2/LTα2β1.** For detection of LTα protein, 96-well ELISA plates were coated overnight with 50 µl of anti-LTαFcmT (clone: S5H3.2.2) antibody diluted 1:200 in coating buffer at 4° C. The next day, the coating solution was discarded and 200 µl PBS 0.05% Tween-20 was added per well incubated for 2 min and again discarded (wash step). 200 µl PBS 5%BSA was added to each well and incubated at room temperature for 1h to block unspecific binding. After blocking, 5 wash steps were performed and afterwards, 50 µl of standard or sample was added per well. The first standard contained 1 µg/ml recombinant LTα in blocking solution, further standards were created by 1:1 serial dilution in blocking solution. Samples were also diluted in blocking buffer. After incubation overnight at 4°C, 5 wash steps were performed and 50 µl of detection antibody (50ng/ml in blocking solution) solution was added per well and incubated for 2 h at room temperature. After 5 wash steps, 50 µl of Avidin-HRP diluted 1:500 in blocking solution was added to each well and incubated for 30 min at room temperature. Again 5 wash steps were performed before adding 50 µl TMB solution to induce a colorimetric reaction. After incubating 15 min in the dark, the reaction was stopped by adding 25 µl of 20% sulphuric acid solution per well. Analysis was done at 450 nm using a Tecan Infinite pro machine.



**Mitochondrial DNA sequencing.** Mitochondrial DNA was amplified from total DNA extracts of tibial anterior muscle tissue using previously established primers<sup>7</sup> (forward: TACGTGATCTGAGTTCAGACCG; reverse: GTAGGACTTTAATCGTTGAACAAAC), resulting in a 16,299 kb PCR product. The long-range PCR products were purified using SPRI beads, and library preparation was performed with the SQK-NBD114.24 kit (ONT). The individual libraries were pooled and sequenced on a MinION sequencer with a Flongle flow cell for 24 hours. The resulting BAM files were aligned to the GRCh38 reference genome using minimap2, and structural variations were identified with Sniffles2.<sup>8</sup>

**RNAseq.** RNA concentration and integrity was verified on a TapeStation (Agilent) and QFX fluorometer (Denovix). Libraries for RNA-Seq were prepared using the QuantSeq FWD V2 kit (Lexogen) according to the manufacturer's protocol. Sequencing was performed on an Illumina NovaSeq6000 (1×100 cycles), generating approximately 8-10 million raw reads per sample. Raw data were demultiplexed and FASTQ files were generated using bcl\_convert. Data were aligned to the GRCh38p14 genome and counted with STAR Aligner<sup>9</sup> and further analyzed and visualized with BioJupies using default parameters<sup>10</sup>. For differential expression (DE) analysis at least three independent biological replicates (n=3) of each genotype were used. Integrated pathway analysis and visualization was done with Pathview<sup>11</sup>

**Analysis of published human RNAseq data.** Data were downloaded from the Gene Expression Omnibus (GEO) database, specifically datasets GSE151757<sup>12</sup> and GSE220915,<sup>13</sup> which include RNA-seq data from Inclusion Body Myositis (IBM) and control samples. Differential expression analysis was performed using DESeq2 in R to identify differentially expressed genes (DEGs) between the two conditions. Adjusted p-values for the comparison between IBM and control samples were calculated using DESeq2.<sup>14</sup> Key gene sets, including lymphotoxin signaling-associated genes and proteostasis-related genes, were highlighted for further investigation. Results were visualized using boxplots.

**Drug treatments.** Mice were subjected to continuous behavioral tests starting from 90 days of age as described above. Treatment was initiated 30 days later, at 120 days of age. The following drugs were applied by the following routes of administration: (1) prednisolone 5mg/kg body weight, orally, diluted in the drinking water; (2) anti-Thy1.2 40mg i.p. three times a week. I.p. injected drugs were diluted in PBS. Controls received the same volume of i.p. injected PBS. Mice of the correct genotypes were randomly allocated to control and treatment group. While drug application was not performed in a blinded manner, behavioural testing and

data analysis were performed in a blinded way. We are not aware of any potential confounders and therefore did not control for them.

**Western blots.** Murine muscle tissue was homogenized using a ceramic mortar and pestle, cooled with liquid nitrogen. The resulting tissue powder was resuspended in a lysis buffer (10 mM HEPES, 0.1 mM MgCl<sub>2</sub>, 1 mM EDTA, containing 1.5% (v/v) protease inhibitor cocktail (Sigma-Aldrich, P8340)). After sonication, TritonX-100 was added to a final concentration of 1% and the samples were incubated for 30 minutes at 4° C on a rocking shaker. After centrifugation (5000 x g, 10 min, 4° C), supernatants were collected and protein concentration was determined using the Micro BCA™ Protein Assay Kit (Thermo Scientific™, 23235).

Five µg of total protein per sample were loaded and separated on a 7-18% gradient SDS-PAGE (80-140 V, 3 h) in running buffer (25 mM TrisBase, 192 mM Glycin, 2% SDS, pH = 8.3). Proteins were transferred onto a PVDF membrane by wet blotting (10 V, overnight, 4°C) in Towbin buffer (25mM TrisBase, 192mM Glycin, 0.1% SDS, 20% MetOH, pH = 8.3). Membranes were incubated at room temperature (RT) for 2 hours in blocking buffer (1% (w/v) Blocking Reagent (Roche, 11096176001), 20mM maleic acid, 30mM NaCl, 0.8 x TBST, 0.01% thimerosal). Incubation with the primary antibodies was performed over night at 4°C at a 1:1000 dilution (anti-α-Tubulin [Sigma-Aldrich, T5168], anti-LC3A/B [Cell Signaling, 12741S], anti-GAPDH [6C5; Abcam, ab8245]). After washing in 1 x TBST membranes were incubated at RT for 2 hours with HRP-conjugated secondary antibodies (goat anti-mouse IgG [1:2000, Invitrogen, 32430], Goat anti-Rabbit IgG [1:1000, Invitrogen, 31460]). After washing in 1x TBST, band detection was performed using the SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Scientific™, 34095) and developed using the iBright™ FL1500 Imaging System (Invitrogen™). The band intensities were analyzed with the iBright™ Image Analysis Software (version 5.2.1).

## References for supplementary methods

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