Figure EV1.
Figure EV1. Analysis of Mfn2 expression in different tissues during aging.

A Mfn2 protein expression was evaluated in tibialis and soleus of young (6-month-old) and old (22-month-old) mice (n = 6 mice per group).
B Mfn2 protein expression was evaluated in heart and liver from young and old mice (n = 6 mice per group).
C Mfn2 protein expression was measured in gastrocnemius muscle from young (6-month-old), middle aged (12-month-old), and old (22-month-old) mice (n = 6 mice per group).
D Mfn2 gene expression was measured in young and old mice (n = 6 mice per group).
E Mfn2 expression was analyzed in polysomal fractions obtained from quadriceps muscle from young and old mice (n = 6 mice per group).
F Expression of mitochondrial dynamics proteins was analyzed in gastrocnemius muscle from young and old control and Mfn2KO mice (n = 6 mice per group).

Data information: Data were expressed as a fold change compared to young control mice and represent mean ± SEM. *P < 0.05 Mfn2KO vs. control mice, *P < 0.05 old vs. young mice.

Figure EV2. Mfn2 deficiency does not alter proteasome-dependent protein degradation but reduces protein synthesis in skeletal muscle.

A mRNA expression of FbxO32 (atrogin-1), Murf1, SMART, FbxO31, and MUSA1 was evaluated by quantitative PCR in gastrocnemius muscle from young and old control and Mfn2KO mice (n = 4–6 mice per group). Data were expressed as a fold change compared to young control mice.
B Levels of K48-linked ubiquitinated proteins were measured in gastrocnemius muscle from young and old control and Mfn2KO mice (n = 4–6 mice per group). Data were normalized by tubulin as a loading control and expressed as a fold change compared to young control mice.
C–E Proteasome activity was measured in gastrocnemius muscle from young and old control and Mfn2KO mice (n = 4–6 mice per group). Data were expressed as a fold change compared to young control mice.
F Protein expression and phosphorylation levels of S6 and 4EBP were determined in gastrocnemius muscle from young and old control and Mfn2KO mice (n = 4–6 mice per group). Data were expressed as a fold change compared to young control mice.
G In vivo protein synthesis was measured by puromycin incorporation in gastrocnemius muscle from young control and Mfn2KO mice (n = 5 mice per group). Data were normalized by tubulin as a loading control and expressed as fold change compared to control mice.

Data information: Data represent mean ± SEM. *P < 0.05 Mfn2KO vs. control mice, *P < 0.05 old vs. young mice.
Figure EV2.

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Figure EV2.
Figure EV3. Mfn2 deficiency inhibits autophagy and mitophagy in skeletal muscle and in C2C12 myotubes.

A mRNA expression of Bnip3, Bnip3L, LC3b, and p62 was evaluated by qPCR in gastrocnemius muscle from young and old control and Mfn2KO mice (n = 4–6 mice per group). Data were expressed as a fold change compared to young control mice.

B Autophagic flux was measured in Mfn2 KD C2C12 myotubes treated with bafilomycin A. Protein expression levels of LC3, p62, NBR1, and BNIIP3 were measured and quantified (n = 3 independent experiments performed in duplicate). Data were normalized by actin as a loading control and expressed as fold change compared to control cells.

C Levels of BNIIP3, LC3, and p62 protein expression were evaluated in mitochondrial fractions from young control and Mfn2KO mice untreated or treated with chloroquine for 5 days (n = 4–5 mice per group). TIM44 was used as a loading control.

D Mitochondrial mass was evaluated by measuring mtDNA copy number in control and Mfn2KD C2C12 myotubes (n = 3 independent experiments performed in triplicate). Data were expressed as a fold change compared to control cells.

E CSA was analyzed in gastrocnemius muscle from young control and Mfn2KO mice untreated or treated with chloroquine as indicated (n = 3 mice per group, 200 fibers per muscle).

F Protein levels of Mfn2 in gastrocnemius muscle of young control and Mfn2KO mice, and Mfn2KO mice injected intramuscularly with adenovirus encoding for Mfn2 (n = 6 muscles per group). Data were normalized by tubulin as a loading control and expressed as a fold change compared to control LacZ-infected mice.

G Autophagy protein expression in young control and Mfn2KO mice, and Mfn2KO mice injected intramuscularly with adenovirus encoding for Mfn2 (n = 6 muscles per group). Data were normalized by tubulin as a loading control and expressed as a fold change compared to control LacZ-infected mice.

H CSA of gastrocnemius muscle from young control and Mfn2KO mice, and Mfn2KO mice injected intramuscularly with adenovirus encoding for Mfn2 (n = 4 muscles per group, 200 fibers per muscle). Data were expressed as percentage of CSA compared to control LacZ-infected mice.

Data information: Data represent mean ± SEM. *P < 0.05 Mfn2KO vs. control mice or Mfn2KD vs. control cells, **P < 0.05 old vs. young mice, treated vs. untreated cells, or treated vs. untreated mice.
Figure EV4. Mfn2 deficiency increases expression of HIF1α target genes and inhibition of autophagy is sufficient to induce HIF1α expression.

A mRNA expression of HIF1α target genes Vegfa, Pdk1, and Pdk4 was measured by qPCR in gastrocnemius muscle from young and old control and Mfn2KO mice (n = 4–6 mice per group). Data were expressed as a fold change compared to young control mice.

B mRNA expression of HIF1α target genes Pdk1 and Pdk4 was measured by qPCR in control and Mfn2KD C2C12 myotubes (n = 3 independent experiments performed in duplicate). Data were expressed as a fold change compared to control cells.

C Mitochondrial mass was evaluated by measuring mtDNA copy number in control and Mfn2KD C2C12 myotubes untreated or treated with NSC-134754 (10 μM for 16 h) (n = 3 independent experiments performed in duplicate). Data were expressed as a fold change compared to control cells.

D Expression of autophagic proteins (BNIP3, LC3II, and Parkin) was measured in mitochondrial-enriched fractions from C2C12 myotubes untreated or treated with bafilomycin A (200 nM, 16 h) (n = 3 independent experiments).

E, F Respiration was measured in C2C12 myotubes untreated or treated with bafilomycin A (200 nM, 16 h), and ATP-coupled respiration (E) and proton leak (F) were calculated (n = 3 independent experiments). Data were expressed as a fold change compared to control cells.

G H2O2 levels were determined in C2C12 myotubes untreated or treated with bafilomycin A (200 nM, 16 h) (n = 3 independent experiments performed in duplicate).

H HIF1α and BNIP3 protein expression was measured in C2C12 myotubes untreated or treated with bafilomycin A (200 nM, 16 h) (n = 3 independent experiments performed in duplicate).

Data information: Data represent mean ± SEM. *P < 0.05 Mfn2KO vs. control mice, Mfn2KD vs. control cells, or treated vs. untreated cells; *P < 0.05 old vs. young mice.
Figure EV5: Mfn2 deficiency-induced increase of HIF1α is dependent on ROS.

A. H2O2 levels were measured in gastrocnemius muscle from control and Mfn2 KO young mice untreated or treated with NAC (1% in drinking water) for 3 weeks (n = 5 mice per group).

B. H2O2 levels were determined in control and Mfn2 KD C2C12 myotubes untreated or treated with NAC (5 mM, 16 h) (n = 3 independent experiments performed in duplicate). Data were expressed as a fold change compared to control cells.

C. HIF1α and BNIP3 protein expression was measured in control and Mfn2 KD C2C12 myotubes untreated or treated with NAC (5 mM, 16 h) (n = 3 independent experiments performed in duplicate).

D. Autophagic flux in C2C12 control and Mfn2 KD myotubes untreated or treated with NAC (5 mM, 16 h). Data were calculated as a fold change between bafilomycin and basal situation, and normalized to control cells (n = 3 independent experiments performed in duplicate).

E. CSA of gastrocnemius muscle of young control and Mfn2 KO untreated or treated with NAC for 3 weeks (n = 5 mice per group, 200 fibers per mice).

Data information: Data represent mean ± SEM. # P < 0.05 Mfn2 KO vs. control mice or Mfn2 KD vs. control cells. * P < 0.05 treated vs. untreated cells, or old vs. young mice.

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