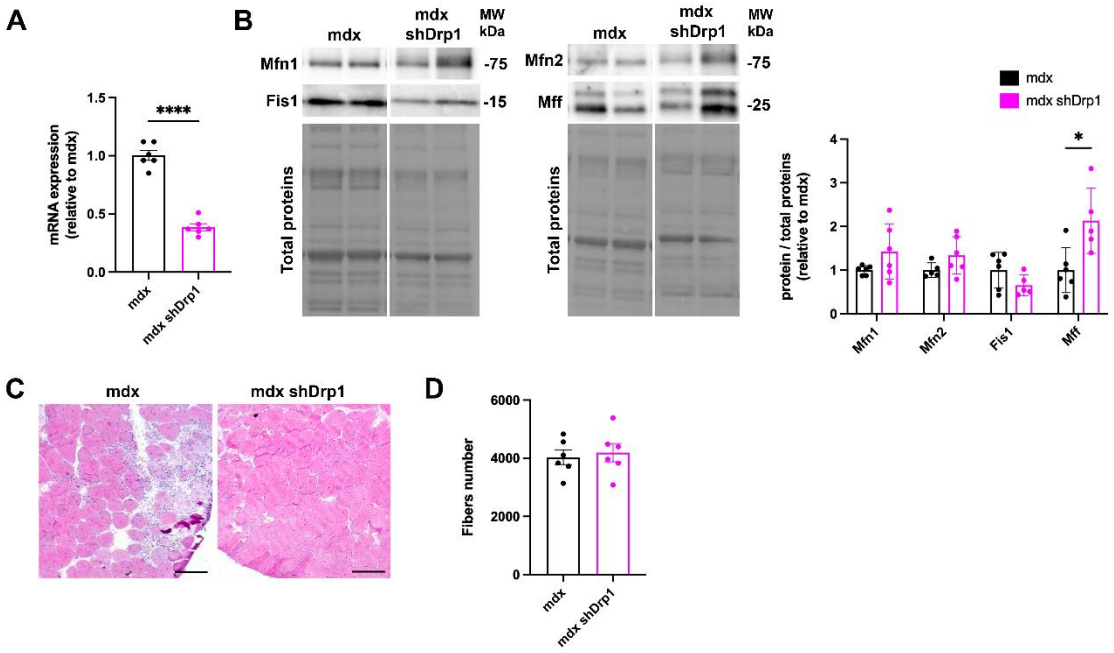

Figure S1. Drp1 knockdown effects on mitochondrial shaping proteins and TA morphology

(A) mRNA expression levels of *Drp1* in TA muscles from *mdx* mice injected with either AAV-shDrp1 (*mdx* shDrp1) or PBS (*mdx*) (n = 6 per group).

(B) Representative western blot images and quantification of mitochondrial dynamics protein levels (Mfn1, Mfn2, Fis1, and Mff) in TA muscles from *mdx* mice injected with either AAV-shDrp1 (*mdx* shDrp1) or PBS (*mdx*) (n ≥ 5 per group). Total protein staining was used for normalization.

(C) Representative images of TA muscle sections from *mdx* mice treated with AAv-shDrp1 (*mdx* shDrp1) or PBS (*mdx*) stained with hematoxylin and eosin (H&E). Scale bars: 200 µm.

(D) Total number of fibers in TA muscle sections from *mdx* mice injected with either AAV-shDrp1 (*mdx* shDrp1) or PBS (*mdx*) (n = 6 per group).

Data are represented as mean ± SEM, ****p < 0.0001. (A, D) Unpaired t-test. (B) Multiple unpaired t-test.

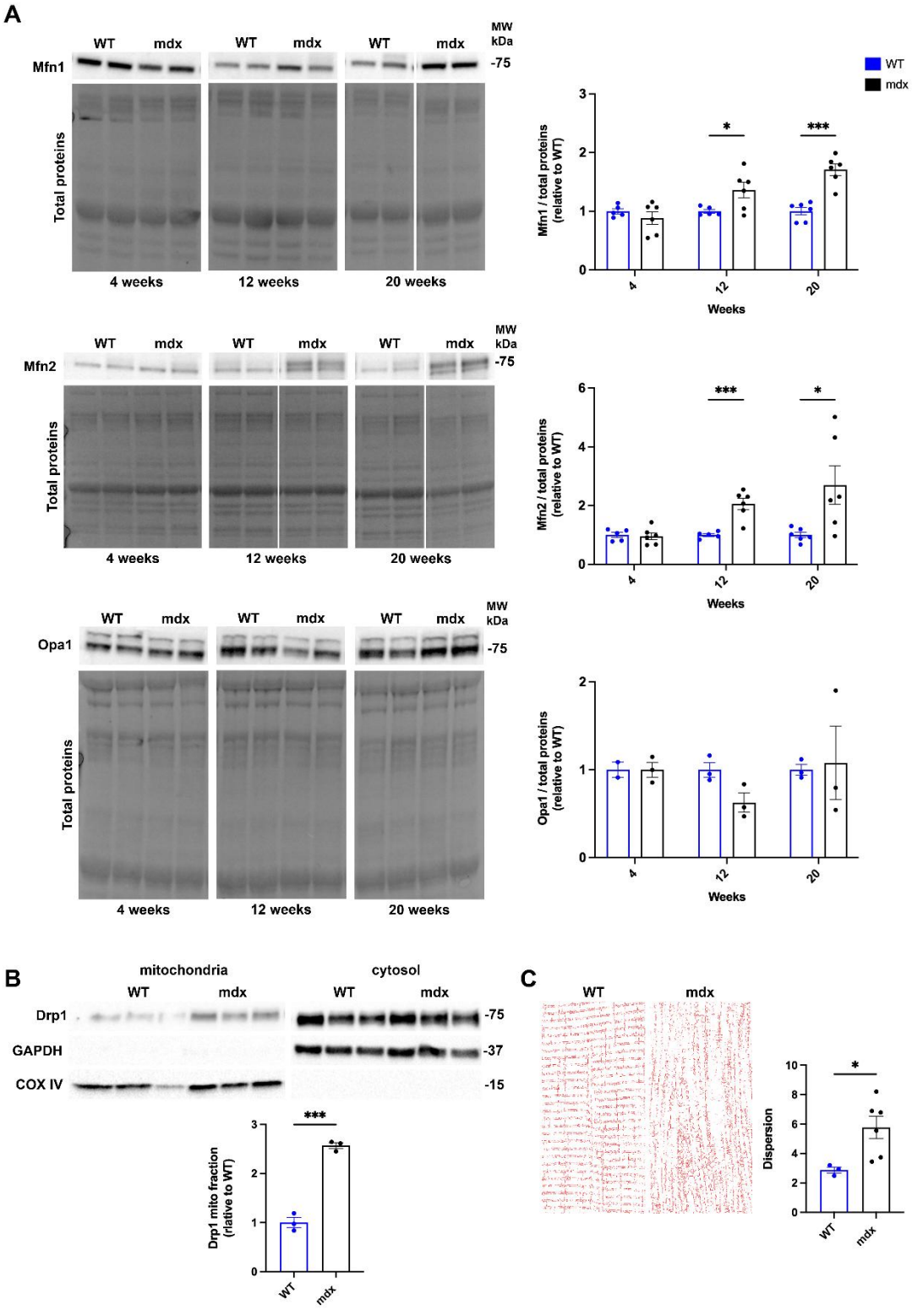

Figure S2. Mitofusins are upregulated during DMD progression, but Drp1 is more active in *mdx* muscles.

(A) Representative western blot images and quantification of mitochondrial shaping protein levels (Mfn1, Mfn2, and Opa1) in GS muscles of WT and *mdx* mice at different disease stages (4, 12, and 20 weeks of age) ($n \geq 3$ per group). Total protein staining was used for normalization.

(B) Representative western blot images of Drp1 protein levels in mitochondrial and cytosolic fractions of GS muscles from 20-week-old WT and *mdx* mice. COX IV and GAPDH have been used as mitochondrial and cytosolic markers, respectively. Quantification of Drp1 protein levels in the mitochondrial fraction, normalized on COX IV, is provided ($n = 3$ per group).

(C) Representative skeletonized images of the mitochondrial network in soleus single fibers from 20-week-old WT and *mdx* mice. Quantification of mitochondrial network dispersion was performed using the Directionality plugin of ImageJ ($n \geq 3$ per group).

Data are represented as mean ± SEM, $*p < 0.05$, $**p < 0.01$, $*** p < 0.001$. (A) Multiple unpaired t-test. (B, C) Unpaired t-test.

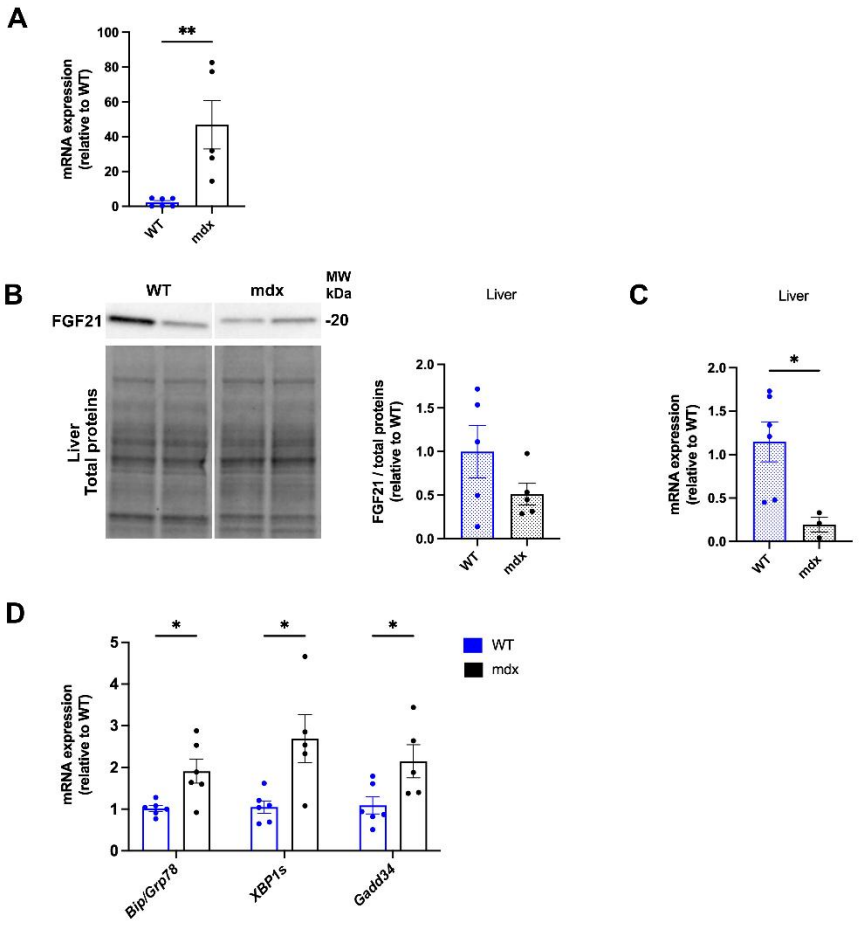

Figure S3. FGF21 is specifically produced by the DMD muscle that also displays ER stress induction.

(A) mRNA expression levels of *FGF21* in GS muscles from 20-week-old WT and *mdx* mice (n ≥ 5 per group).

(B) Representative western blot images and quantification of FGF21 protein levels in liver homogenates from 20-week-old WT and *mdx* mice (n = 5 per group). Total protein staining was used for normalization.

(C) mRNA expression levels of *FGF21* in liver homogenates from 20-week-old WT and *mdx* mice (n ≥ 3 per group).

(D) mRNA expression levels of ER stress markers (*Bip/Grp78, XBP1 splicing, Gadd34*) in GS muscles from 20-week-old WT and *mdx* mice (n ≥ 5 per group).

Data are represented as mean ± SEM, *p < 0.05, **p < 0.01. (A) Mann-Whitney test. (B, C) Unpaired t-test. (D) Multiple unpaired t-test.

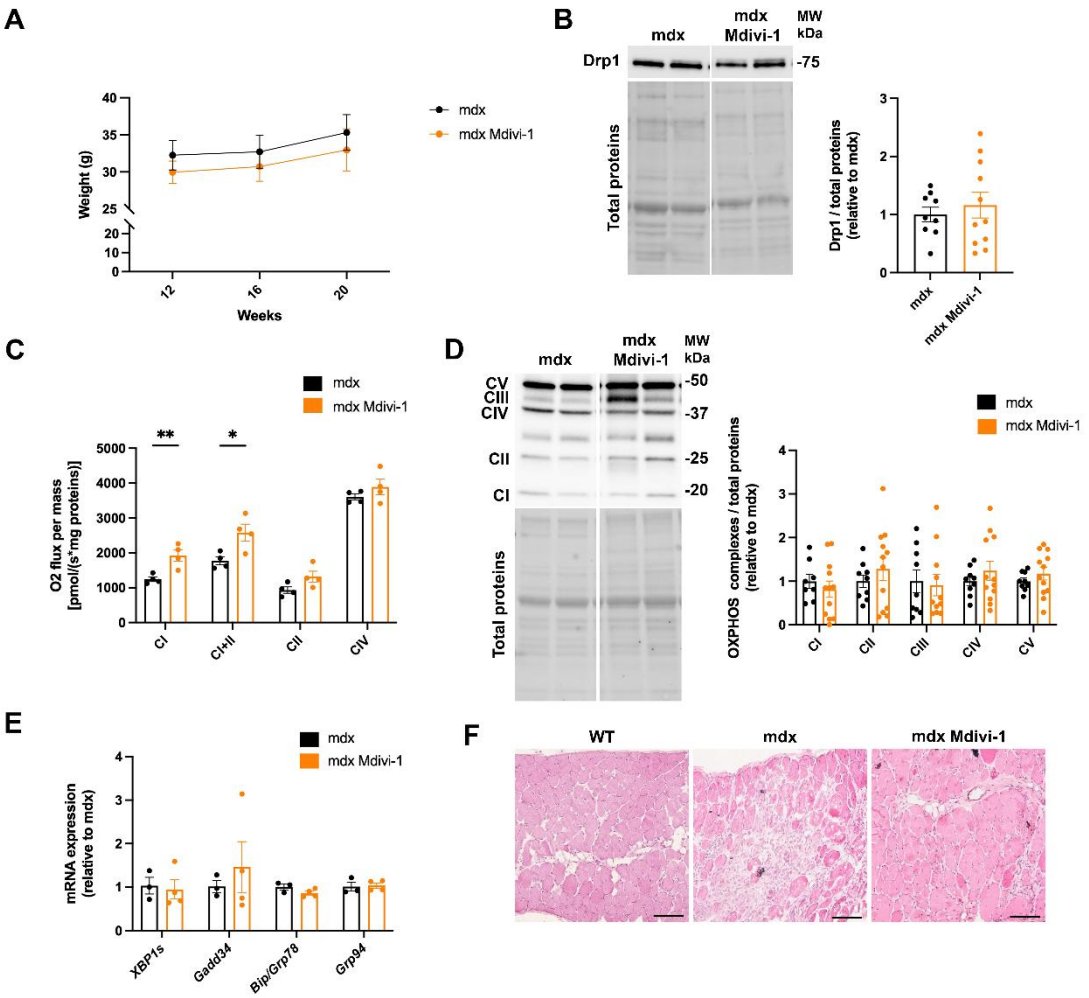

Figure S4. Mdivi-1 improves mitochondrial metabolism but does not affect the levels of ER stress markers.

(A) Weight of *mdx* mice treated with either mdivi-1 (*mdx* Mdivi-1) or vehicle (*mdx*) at the beginning, in the middle, and at the end of the treatment (12, 16, and 20 weeks).

(B) Representative western blot images and quantification of Drp1 protein levels in GS muscles from *mdx* mice treated with either mdivi-1 (*mdx* Mdivi-1) or vehicle (*mdx*) ($n \geq 9$ per group). Total protein staining was used for normalization.

(C) Oxygen consumption values of isolated mitochondria from TA fresh muscles of *mdx* mice treated with either mdivi-1 (*mdx* Mdivi-1) or vehicle (*mdx*) ($n = 4$ per group). Oxygen flux was normalized on total protein content (mg).

(D) Representative western blot images and quantification of OXPHOS complexes (CI, CII, CIII, CIV, and CV) protein levels in GS muscles from *mdx* mice treated with either mdivi-1 (*mdx* Mdivi-1) or vehicle (*mdx*) ($n \geq 8$ per group). Total protein staining was used for normalization.

(E) mRNA expression levels of ER stress markers (*XBP1 splicing, Gadd34, Bip/Grp78, Grp94*) in GS muscles from *mdx* mice treated with either mdivi-1 (*mdx* Mdivi-1) or vehicle (*mdx*) ($n \geq 3$ per group).
(F) Representative hematoxylin and eosin images of DP muscle sections from *mdx* mice treated with either mdivi-1 (*mdx* Mdivi-1) or vehicle (*mdx*) and from age-matched WT mice. Scale bars: 200 μm.
Data are represented as mean ± SEM, *$p < 0.05$, **$p < 0.01$. (A, C-E) Multiple unpaired t-test. (B) Unpaired t-test.

Table 1: List of primers used for RTqPCR (F: forward; R: reverse).

Gene	Primer sequence
FGF21	F: 5'-CTGGGGGTCTACCAAGCATA-3' R: 5'-CACCCAGGATTTGAATGACC-3'
Grp94	F: 5'-CTCAGAAGACGCAGAAGACTCA -3' R: 5'-AAAACTTCACATTCCCTCTCCA-3'
BiP/Grp78	F: 5'-TGTGGTACCCACCAAGAAGTC-3' R: 5'-TCCAGCTGTCACTCGGAGAAT-3'
36B4	F: 5'-AGGATATGGGATTCGGTCTCTTC-3' R: 5'-TCATCCTGCTTAAGTGAACAAACT-3'
COL1A1	F: 5'-GCTCCTCTTAGGGGCCACT-3' R: 5'-CCACGTCTCACCATTGGGG-3'
CTGF	F: 5'-GGGCCTCTTCTGCGATTTC-3' R: 5'-ATCCAGGCAAGTGCATTGGTA-3'
α-SMA	F: 5'-GTCCCAGACATCAGGGAGTAA-3' R: 5'-TCGGATACTTCAGCGTCAGGA-3'
TGF-β	F: 5'-AAACGGAAGCGCATCGAA -3' R: 5'-GGGACTGGCGAGCCTTAGTT-3'
KLF4	F: 5'-CAGTGGTAAGGTTTCTCGCC-3' R: 5'-GCCACCCACACTTGTGACTA-3'
C/EBP-α	F: 5'-TATGACATCAGCGCCTACATCGA-3' R: 5'-GTCGGCTGTGCTGGAAGAG-3'
C/EBP-β	F: 5'-GCCAAGAAGACGGTGGACAA -3' R: 5'-ACAAGTTCCGCAGGGTGCT-3'
Med23	F: 5'-TCGGAAAATCATTGGAGGAG-3' R: 5'-CAATAGGCAGGCATTTCGTT-3'
PPAR-γ	F: 5'-GCAGAATAAAAGGTGCCACAG-3' R: 5'-CGTCTCCGTGACGAAGTCAA-3'
Drp1	F: 5'-GCTGGATCACGGGACAAGTTAA-3' R: 5'-TGCCTGTTGTTGGTTCCTGAC-3'
Gadd34	F: 5'-GAGGGACGCCCACAACTTC-3' R: 5'-TTACCAGAGACAGGGGTAGGT-3'
XBP1s	F: 5'-GAGTCCGCAGCAGGTG-3' R: 5'-GTGTCAGAGTCCATGGGA-3'
hDrp1	F: 5'-CAAAGCAGTTTGCCTGTGGA-3' R: 5'-TCTTGGAGGACTATGGCAGC-3'

Table 2: List of antibodies used and dilution applied in western blot (WB) and immunofluorescence (IF).

Epitope	Product name (catalogue number)	Manufacturer	Species	Dilution
Drp1	611113 (clone8-DLP1)	BD Transduction Laboratories	M	1: 1000 WB
GAPDH	sc-25778	Santa Cruz Biotechnology	R	1:1000 WB
Coll3A1	sc-8780 (S-17)	Santa Cruz Biotechnology	R	1:1000 WB
Fis1	Ab-84484	Immunological Science	R	1: 1000 WB
Mff	Ab-84491	Immunological Science	R	1: 1000 WB
eIF2α	9722	Cell signaling	R	1: 1000 WB
p-eIF2α	3597S (Phospho-eIF2α (Ser51) (119a11))	Cell signaling	R	1: 1000 WB
CHOP	Ab114119 (clone-9C8)	Abcam	M	1:2000 WB
ClpP	WH0008192M1	Merck	M	1:1000 WB
HSP60	sc-59567 (HSP 60 (LK1))	Santa Cruz Biotechnology	M	1:500 WB
FGF21	Ab171941 (clone-EPR8314(2))	Abcam	R	1:1000 WB
Mfn1	H00055669-M04 (clone-3C9)	Abnova Corporation	M	1:1000 WB
Mfn2	sc-100560 (Mfn2 XX-1)	Santa Cruz Biotechnology	M	1:1000 WB
Mff	Ab-84491	Immunological Science	R	1:1000 WB
Opa1	612606	BD Transduction Laboratories	M	1:1000 WB
COX IV	Ab-14744 (clone-20E8C12)	Abcam	M	1:2000 WB
OxPhos	45-8099 OxPhos Rodent WB antibody cocktail	Invitrogen	M	1:1000 WB
Laminin	L9393	Merck, Darmstadt, Germany	R	1:250 IF
Laminin	MA1-06100 (clone-A5)	Invitrogen	Rat	1:200 IF
MYH3	sc-53091 MYH3 (F1.652)	Santa Cruz Biotechnology	M	1:100 IF
Perilipin A/B	P1873	Merck	R	1:200 IF
CD45	130-102-491 (CD45-FITC)	Miltenyi-Biotec	Rat	1:200 IF
MHC	MF-20	Developmental Studies Hybridoma Bank	M	1:50 IF