Expanded View Figures

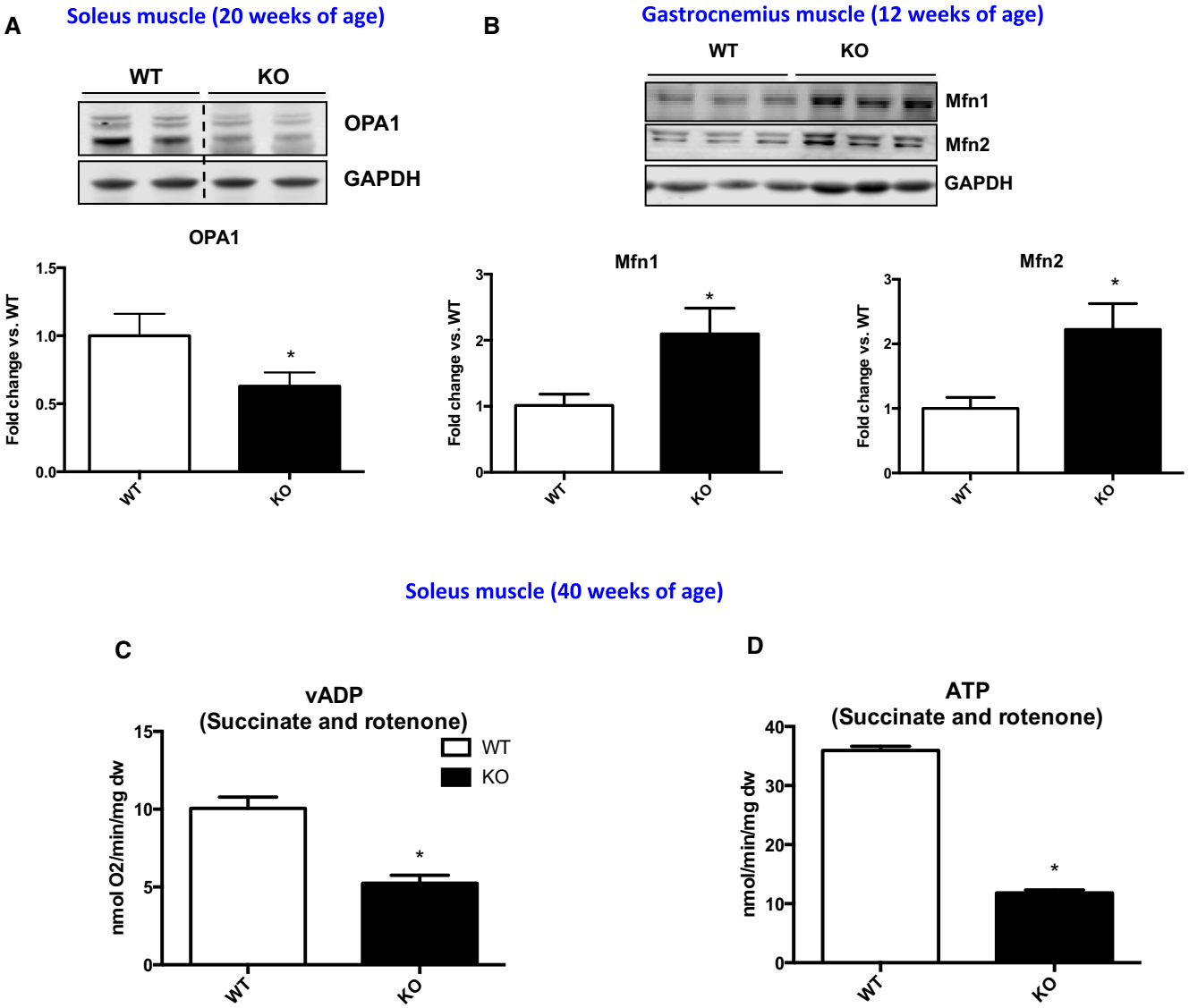

Figure EV1. Mitochondrial dynamics proteins and mitochondrial oxygen consumption.

A, B Representative immunoblots and densitometric quantification in muscle homogenates from WT and mOPA1 KO mice. (A) OPA1 levels in soleus muscle normalized to GAPDH (n = 3). (B) Mfn1 and Mfn2 normalized to GAPDH (n = 4–5).

C Maximally stimulated succinate-supported mitochondrial respirations in soleus from 40-week-old WT and mOPA1 KO (KO) mice (n = 5).

D Succinate-supported ATP synthesis rates in soleus from 40-week-old WT and mOPA1 KO (KO) mice (n = 5).

Data information: Data are expressed as means ± SEM. Significant differences were determined by Student's t-test, using a significance level of $P < 0.05$. (*) Significantly different vs. WT mice.
Source data are available online for this figure.

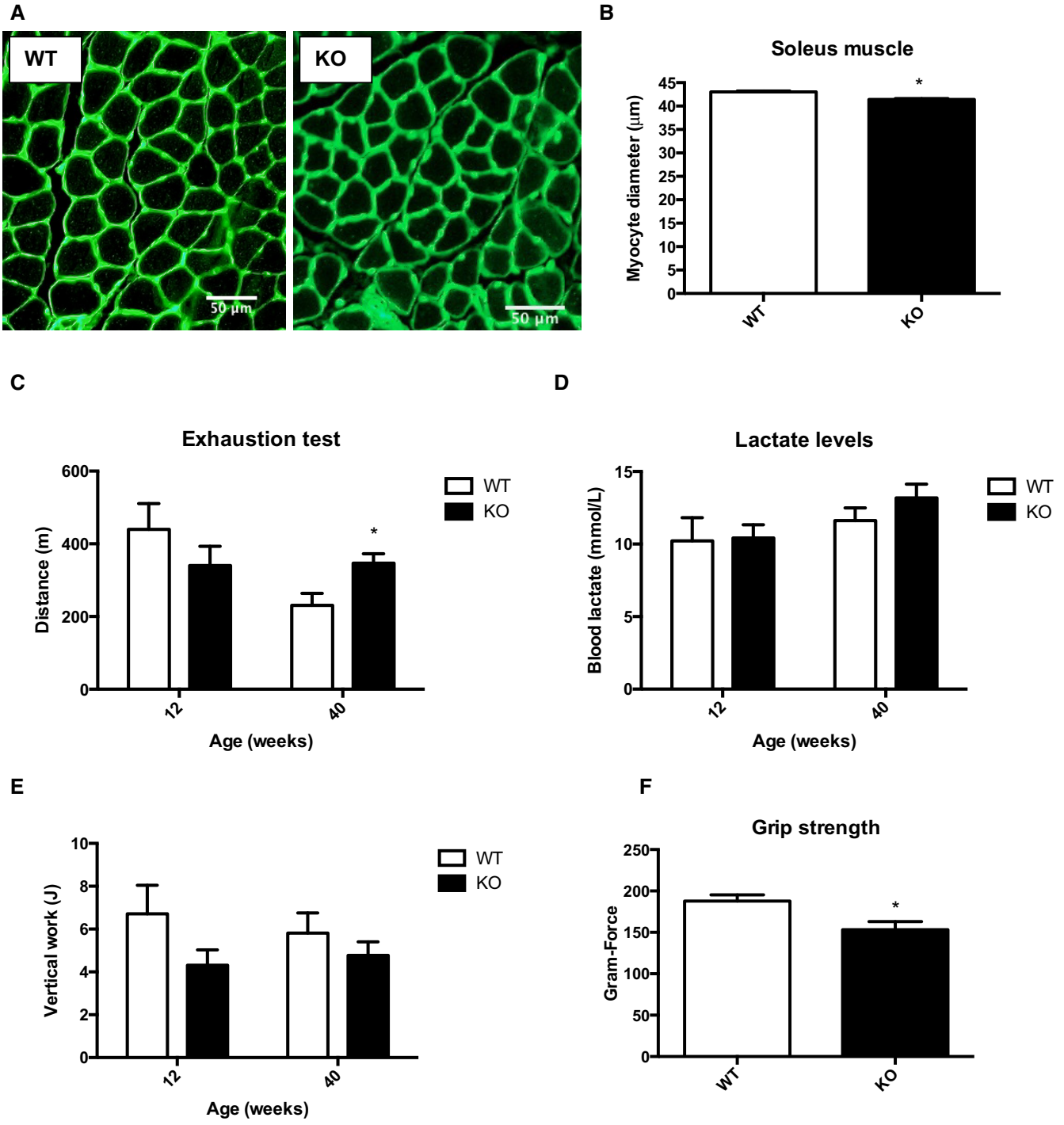

Figure EV2. Assessment of overall muscle health of mOPA1 KO mice.

A Cross sections of soleus muscle from 20-week-old mice stained with wheat-germ agglutinin (WGA). Scale bar = 50 µm.
B Measurements of muscle fiber diameter in soleus muscle from 20-week-old mice (n = 4).
C Exhaustion test in 20- and 40-week-old WT and mOPA1 KO mice (n = 4–9).
D Blood lactate after the exhaustion test (n = 4–9).
E Measurements of vertical work performed by 20- and 40-week-old mice during the exhaustion test (n = 4–9).
F Measurements of grip strength in 40-week-old mice (n = 5).

Data information: Data are expressed as means ± SEM. Significant differences were determined by Student's t-test, using a significance level of $P < 0.05$. (*) Significantly different vs. WT mice.
Source data are available online for this figure.

Figure EV3. Metabolic parameters in mOPA1 KO mice.

A Triglycerides levels in mice fed either a control or a HFD for 12 weeks (n = 5–6).
B Fasting triglyceride levels in the serum of mice fed either a control or a HFD for 12 weeks (n = 5–8).
C *IL-15 and IL-6* mRNA expression in gastrocnemius muscle of 20-week-old WT and mOPA1 KO mice (n = 4–5).
D Representative immunoblot and densitometric quantification of OPA1 and FGF21 normalized to GAPDH in soleus muscle of 20-week-old mice (n = 3).
E Representative immunoblot and densitometric quantification of PGC-1α and UCP1 normalized to GAPDH in BAT of 20-week-old mice (n = 4).
F Representative immunoblot and densitometric quantification of PGC-1α and UCP1 normalized to GAPDH in sc-WAT of 20-week-old mice (n = 3–4). Data are represented as fold change vs. WT mice.

Data information: Data are expressed as means ± SEM. Significant differences were determined by Student's t-test (D, F), using a significance level of $P < 0.05$ or by ANOVA followed by Tukey multiple comparison test (A, B), using a significance level of $P < 0.05$. (*) Significantly different vs. WT mice. (*) vs. WT Cont (#) vs. WT HFD. Source data are available online for this figure.

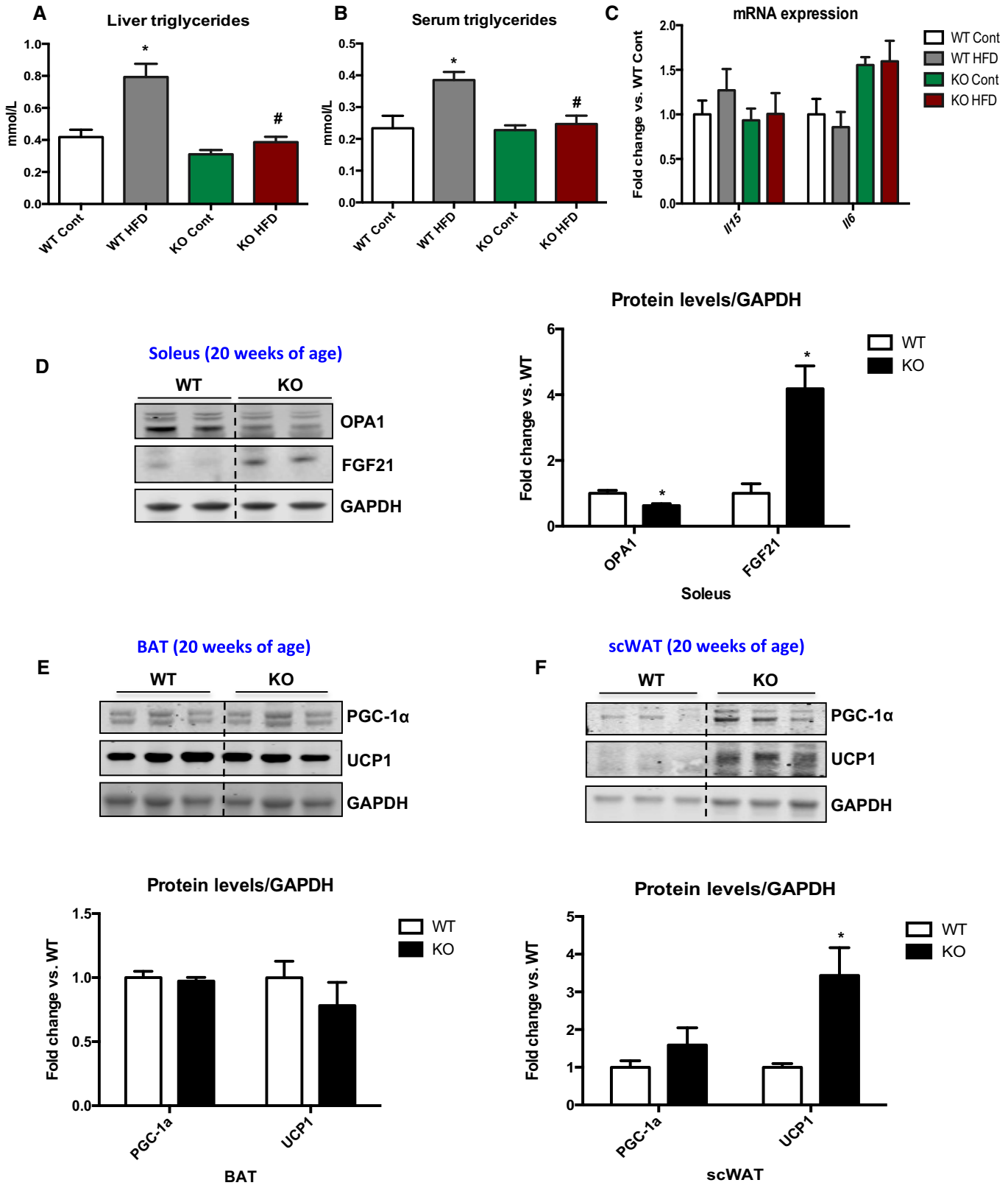

Figure EV3.

Figure EV4. Assessment of overall mitochondrial function and muscle health in mOPA1/FGF21 DKO mice.

A Representative immunoblots and densitometric quantification of OPA1 normalized to GAPDH in gastrocnemius muscle from 12-week-old WT and mOPA1/FGF21 KO mice (n = 3).
B BN-PAGE in gastrocnemius muscle of 12-week-old mice (n = 4).
C mtDNA copy number in gastrocnemius muscle of 12-week-old mice (n = 3–4).
D Maximally stimulated succinate-supported mitochondrial respirations in soleus from 20-week-old mice (n = 4–6).
E Succinate-supported ATP synthesis rates in soleus from 20-week-old mice (n = 4–5).
F Maximally stimulated succinate-supported mitochondrial respirations in soleus from 40-week-old mice (n = 5).
G Measurements of grip strength in 40-week-old mice (n = 3).
H Body weight and body composition in 20-week-old WT and DKO mice (n = 5–9).
I Cross sections of gastrocnemius muscle from 20-week-old mice stained with wheat-germ agglutinin (WGA). Scale bar = 50 μm.
J Measurements of muscle fiber diameter in gastrocnemius muscle from 20-week-old mice (n = 3).

Data information: Data are expressed as means ± SEM. Significant differences were determined by Student's t-test, using a significance level of $P < 0.05$. (*) Significantly different vs. WT mice.
Source data are available online for this figure.

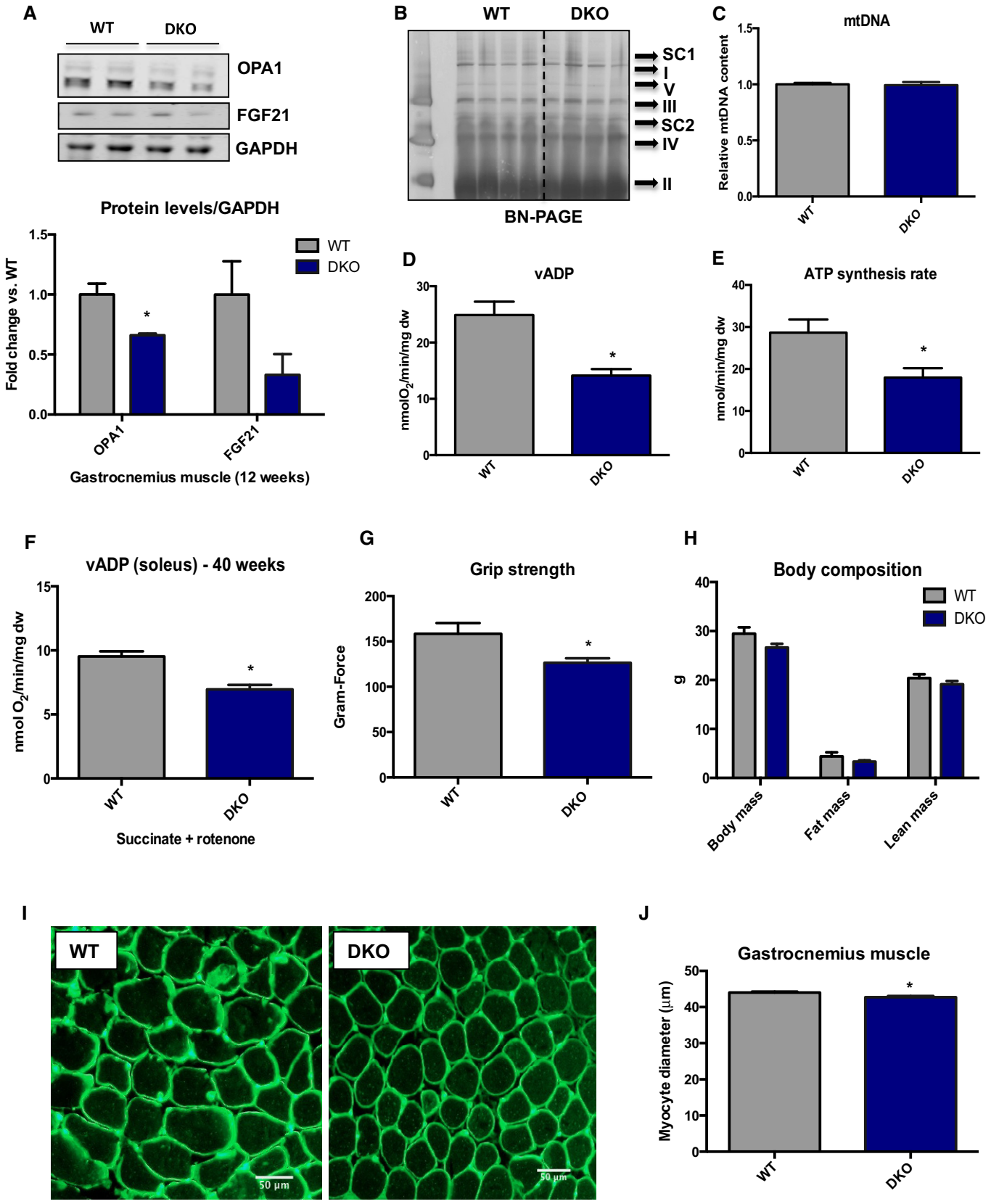

Figure EV4.

Figure EV5. Mitochondrial stress and ER stress in C2C12 myotubes.

A FGF21 protein levels measured by immunoblot, in cell lysates from C2C12 myotubes treated either with vehicle, oligomycin, or tunicamycin ± PBA for 8 h. Data are represented as fold change vs. vehicle normalized to GAPDH (n = 4–8).

B FGF21 in the media secreted from C2C12 myotubes treated either with vehicle, oligomycin, or tunicamycin ± PBA for 8 h (n = 3).

C mRNA expression of *Fgf21*, *Atf4*, and *BiP* in C2C12 myotubes treated with vehicle or oligomycin for 8 h (data are expressed as fold change vs. vehicle) (n = 4–6).

D Western blot analysis and densitometric quantification of phosphorylated AMPK normalized to total AMPK in C2C12 cells treated with vehicle or AICAR for 12 h (n = 4).

E FGF21 mRNA expression in C2C12 cells treated with vehicle or AICAR for 12 h (n = 3–4). Data are represented as fold change vs. vehicle.

Data information: Data are expressed as means ± SEM. Significant differences were determined by Student's t-test (C, D), using a significance level of $P < 0.05$ or by ANOVA followed by Tukey multiple comparison test (A, B), using a significance level of $P < 0.05$. (*) Significantly different vs. vehicle. (*) and (#) vs. oligomycin or tunicamycin.
Source data are available online for this figure.

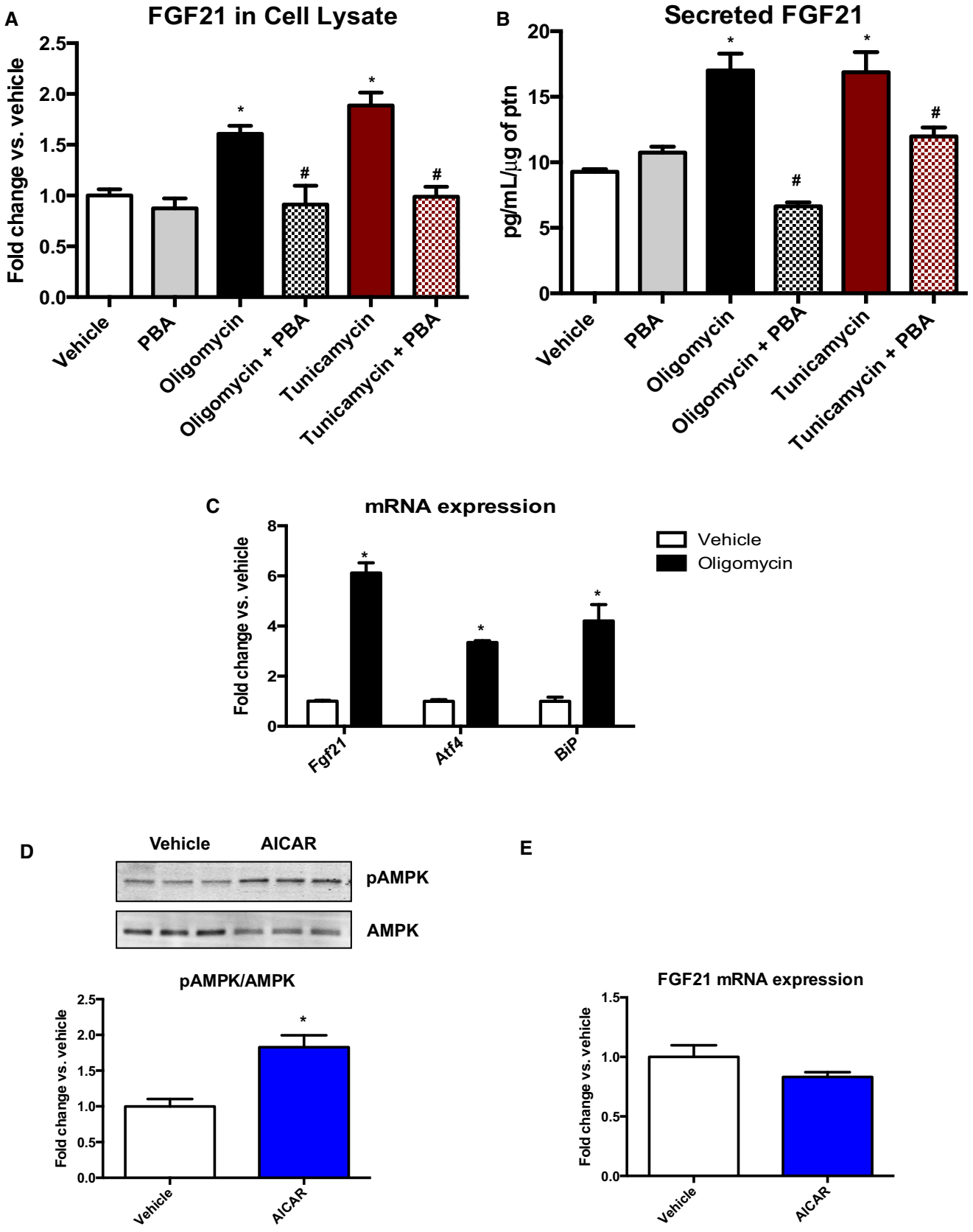

Figure EV5.