SUPPLEMENTAL MATERIAL

Data S1. Supplemental Methods

Preparation of muscle tissue extracts and immunodetection of selected markers of mitochondrial quality control

Whole-tissue extracts were prepared from snap-frozen gastrocnemius muscle samples using an extraction buffer (20 mM HEPES, pH 7.4, 2 mM EGTA, 1% Triton X-100, 2% glycerol, 50 mM β-glycerophosphate, 1× Halt-protease, and phosphatase inhibitor cocktail). Briefly, 20-30 mg of muscle tissue were immersed in a pre-cooled vial containing zirconium beads (Ø 3 mm) and extraction buffer (1:20 w/v), placed in a BeadBug homogenizer (Benchmark Scientific, Sayreville, NJ), and homogenized 5 times at setting 4,000 for 30 s each, with intermittent 1-min cooling on ice. Afterwards, the cleared homogenate was sonicated 10 times for ~3 s each, followed by centrifugation at 10,000 $\times g$ for 10 min at 4 °C. The resulting supernatant was collected and total protein content was determined via the Bradford colorimetric assay. Samples were subsequently diluted in Laemmli sample buffer (Bio-Rad, Hercules, CA) supplemented with β-mercaptoethanol (Bio-Rad) for Western blot, or sample buffer and Fluorescent Master Mix (both from ProteinSimple, San Jose, CA) for the Jess system (ProteinSimple), and proteins were denatured at 95 °C for 5 min. For Western blot immunodetection, 50 µg of protein were loaded onto a 4–20% Criterion TGX Precast Midi Protein Gel (Bio-Rad), and separated in a Bio-Rad running buffer. Separated proteins were transferred onto a polyvinylidenefluoride or a nitrocellulose membrane (0.2 µm; Bio-Rad) using a Bio-Rad Trans-Blot Turbo Transfer System and Transfer buffer. The membranes were reversibly stained with Ponceau S (MilliporeSigma, Burlington, MA), imaged with a Bio-Rad ChemiDoc XRS+ imager to visualize total protein, and subsequently blocked for 60 min at room temperature with 5% Blocking-grade Blocker (Bio-Rad) or 5% bovine serum albumin (MilliporeSigma) in Tris-buffered saline (Bio-Rad) with 0.05% tween-

20 (Bio-Rad). Sample protein concentrations of 0.25 and 0.75 µg/µL were loaded on the Jess

separation module (12 to 230 kDa; ProteinSimple). The following commercially available

primary antibodies and dilutions were used: microtubule-associated protein 1A/1B-light

chain 3 (LC3A/B, Cell Signaling, Danvers, MA, #4108; 1:500), p62 (MilliporeSigma,

#P0067; 1:600), phosphatase and tensin homolog-induced kinase 1 (PINK1, Novus

Biologicals, Centennial, CO, #NB100-644SS; 1:50), Parkin (Abcam, Cambridge, MA,

#ab77924; 1:100), nuclear respiratory factor 1 (NRF1, Santa Cruz Biotechnologies, Dallas,

TX, #sc-101102; 1:50), PGC-1α (MilliporeSigma, #516557; 1:25), mitochondrial

transcription factor A (TFAM, Novus Biologicals #NBP1-71648SS; 1:400), and ETC

complexes I to V (Total OXPHOS antibody cocktail, Abcam #ab110411; 1:500). Secondary

antibodies applied in the Jess system were horseradish peroxidase (HRP)-conjugated anti-

mouse or anti-rabbit IgG from ProteinSimple. HRP-conjugated secondary antibodies for

traditional Western Blot immunodetection were purchased from Cell Signaling (anti-rabbit

IgG #7074, 1:5,000; anti-mouse IgG #7076, 1:5,000). All other reagents and supplies used

for the automated procedures on the Jess system were purchased from ProteinSimple or from

Bio-Rad for traditional Western blots. The optimal dilution of each antibody was determined

against a calibration curve of serial homogenate dilutions to ensure linearity of signal

quantification. The quantification of electropherograms for the Jess system was accomplished

with Compass for SW software (v4.1.0; ProteinSimple) using a Gaussian peak fit or dropped

lines distribution for area under the curve. The peak area for each capillary was normalized to

total protein using the system's Protein Normalization capability and reagents for the Jess

system. For Western blot, protein content was quantified using the Image Lab 6.0 software

(Bio-Rad) based on the spot density of target bands acquired with a ChemiDoc XRS+ imager

(Bio-Rad). The spot density of target bands was normalized to the amount of protein loaded

in each lane as determined by densitometric analysis of the corresponding Ponceau S-stained

membranes.[39,40]

Table S1. Correlation analysis between markers of mitochondrial quality control in gastrocnemius muscle samples and measures of physical performance in participants with and without peripheral artery disease according to disease severity

	Moderate to severe PAD (ABI <0.60)				Mild PAD (ABI 0.60–0.90)				No PAD			
	6MWT (n=15)	4MGS usual (n=15)	4MGS fast (n=15)	SPPB score (n=15)	6MWT (n=29)	4MGS usual (n=28)	4MGS fast (n=28)	SPPB score (n=28)	6MWT (n=23)	4MGS usual (n=23)	4MGS fast (n=23)	SPPB score (n=22)
LC3A/B I	0.188 $P=0.502$	−0.057 $P=0.841$	−0.056 $P=0.843$	−0.076 $P=0.787$	−0.086 $P=0.659$	0.293 $P=0.130$	0.190 $P=0.333$	0.156 $P=0.427$	−0.686 $P<0.001$	−0.675 $P<0.001$	−0.575 $P=0.004$	−0.568 $P=0.006$
LC3A/B II	0.402 $P=0.138$	0.272 $P=0.326$	0.172 $P=0.539$	0.081 $P=0.773$	0.024 $P=0.902$	0.278 $P=0.153$	0.147 $P=0.456$	0.252 $P=0.196$	−0.438 $P=0.037$	−0.484 $P=0.019$	−0.340 $P=0.112$	−0.309 $P=0.161$
LC3 II/LC3 I	0.325 $P=0.238$	0.354 $P=0.195$	0.239 $P=0.391$	0.114 $P=0.687$	0.151 $P=0.434$	0.159 $P=0.418$	0.0045 $P=0.982$	0.214 $P=0.275$	0.286 $P=0.187$	0.179 $P=0.415$	0.268 $P=0.216$	0.289 $P=0.192$
p62	−0.133 $P=0.637$	−0.092 $P=0.745$	0.017 $P=0.953$	−0.018 $P=0.949$	−0.179 $P=0.352$	−0.235 $P=0.229$	−0.218 $P=0.265$	−0.058 $P=0.769$	−0.254 $P=0.242$	−0.297 $P=0.168$	−0.270 $P=0.214$	−0.108 $P=0.631$
PINK1	0.131 $P=0.643$	0.133 $P=0.637$	−0.017 $P=0.953$	−0.050 $P=0.860$	−0.199 $P=0.300$	0.196 $P=0.316$	0.035 $P=0.860$	0.103 $P=0.602$	−0.277 $P=0.200$	−0.410 $P=0.052$	−0.284 $P=0.190$	−0.156 $P=0.487$
PINK1-cleaved	0.118 $P=0.675$	−0.123 $P=0.662$	−0.235 $P=0.400$	−0.150 $P=0.594$	−0.089 $P=0.647$	−0.138 $P=0.485$	−0.177 $P=0.369$	−0.0045 $P=0.982$	0.377 $P=0.076$	0.321 $P=0.135$	0.419 $P=0.047$	0.344 $P=0.116$

Parkin	−0.320 P=0.245	−0.485 P=0.067	−0.574 P=0.025	−0.474 P=0.074	0.023 P=0.906	−0.160 P=0.415	−0.138 P=0.482	0.048 P=0.808	0.049 P=0.826	−0.121 P=0.581	−0.027 P=0.903	0.001 P=0.998
NRF1	−0.113 P=0.689	0.092 P=0.746	0.024 P=0.932	−0.182 P=0.516	−0.227 P=0.236	0.143 P=0.469	0.066 P=0.738	0.118 P=0.551	−0.351 P=0.101	−0.525 P=0.010	−0.387 P=0.068	−0.235 P=0.293
PGC-1α	−0.219 P=0.432	−0.646 P=0.009	−0.553 P=0.033	−0.309 P=0.262	−0.254 P=0.184	−0.045 P=0.818	−0.034 P=0.866	0.026 P=0.895	−0.323 P=0.133	−0.451 P=0.031	−0.318 P=0.139	−0.277 P=0.211
TFAM	0.169 P=0.548	−0.257 P=0.355	−0.326 P=0.236	−0.232 P=0.406	−0.245 P=0.201	−0.167 P=0.397	−0.250 P=0.200	−0.102 P=0.604	0.264 P=0.223	0.175 P=0.425	0.272 P=0.209	0.178 P=0.429
Complex I	0.032 P=0.911	0.161 P=0.566	0.371 P=0.173	0.331 P=0.228	0.131 P=0.499	0.258 P=0.184	0.079 P=0.688	0.142 P=0.470	0.541 P=0.008	0.477 P=0.021	0.628 P=0.001	0.337 P=0.125
Complex II	0.042 P=0.882	0.198 P=0.479	0.543 P=0.037	0.411 P=0.128	0.101 P=0.603	0.088 P=0.658	0.009 P=0.963	0.125 P=0.526	0.623 P=0.002	0.532 P=0.009	0.640 P=0.001	0.439 P=0.041
Complex III	0.148 P=0.600	0.418 P=0.121	0.693 P=0.004	0.586 P=0.022	0.188 P=0.330	0.200 P=0.307	0.118 P=0.549	0.176 P=0.369	0.530 P=0.009	0.452 P=0.030	0.554 P=0.006	0.352 P=0.108
Complex IV	0.078 P=0.781	0.0001 P=1.000	0.160 P=0.570	0.062 P=0.827	−0.147 P=0.448	0.105 P=0.596	−0.207 P=0.291	0.068 P=0.730	0.557 P=0.006	0.510 P=0.013	0.660 P=0.001	0.379 P=0.082
Complex V	0.178 P=0.525	0.502 P=0.057	0.735 P=0.002	0.509 P=0.053	0.126 P=0.515	0.047 P=0.813	0.068 P=0.731	0.097 P=0.624	0.597 P=0.003	0.500 P=0.015	0.590 P=0.003	0.364 P=0.096

For each variable pair, the Pearson's correlation coefficient and the corresponding *P* value are shown. 4MGS fast indicates 4-m gait speed at fast pace; 4MGS usual, 4-m gait speed at usual pace; 6MWT, 6-min walking test; ABI, ankle-brachial index; LC3B, microtubule-associated protein 1A/1B-light chain 3 non-lipidated (I), and lipidated (II) forms; NRF1, nuclear respiratory factor 1; PAD, peripheral artery disease; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; PINK1, phosphatase and tensin homolog-induced kinase 1; SPPB, short physical performance battery; and TFAM, mitochondrial transcription factor A.