Supplemental Figure 1. Analysis of meteorin-like (Metrnl) gene expression in skeletal muscle biopsies after eight weeks of no exercise (control, n=9), endurance training (n=10), resistance training (n=10), or combined training (n=5). Gene expression was analyzed by qPCR. *p < 0.05 and ***p < 0.0001. All data are presented as mean ± SEM.
SUPPLEMENTAL METHODS

Outpatient Visit. Body composition was determined using dual energy x-ray absorptiometry (DEXA) (DPX-IQ; Lunar, Madison) (1) and 1-cut computed tomography of the mid-thigh (2). Cardiorespiratory fitness ($\text{VO}_2\text{peak}$) was measured during an incremental exercise test on an electronically braked cycle ergometer using indirect calorimetry. Muscle strength was measured using 1-repetition maximum (1-RM) for the leg- and chest-press in the Dan Abraham Healthy Living Center. Participants received an Actigraph accelerometer to wear for the 7 days. The accelerometer data were adjusted using a validated wear-time algorithm (3), with a minimum of 10 hours of wear-time data per day for a valid wear day, and a minimum of 4 valid wear days to be included as described previously (4). Two subjects were missing a mid-thigh computed tomography scans, one subject had an incomplete follow-up $\text{VO}_2\text{peak}$ assessment, and a few subjects were missing 1-RM data as indicated.

Inpatient Visit. Participants received a standardized weight-maintenance diet (50:20:30 % energy for carbohydrate:protein:fat) for the three days preceding their inpatient study, and were admitted to the CRU at ~1700 h on the third day of their standardized diet and remained until the completion of the inpatient study day following afternoon.

Muscle Biopsies. Vastus lateralis muscle biopsy samples (300-500 mg) were obtained under local anesthesia (lidocaine, 2%) using a modified Bergstrom needle (Cadence Scientific, Staunton, VA), as previously described (5). A portion of the muscle biopsy sample was immediately placed in ice-cold relaxation and biopsy preservation buffer (BIOPS: 10 mM Ca$^{++}$-EGTA, 0.1 µM free Ca$^{++}$, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM MgCl$_2$, 5.77 mM ATP, 15 mM phosphocreatine) for measurement of mitochondrial oxidative capacity using high-resolution respirometry.

Mitochondrial Isolation. Mitochondria were isolated from the fresh muscle by differential centrifugation, as described in detail elsewhere (6). The final mitochondrial pellet was re-suspended in mitochondrial respiration buffer (MiR05: 0.5 mM EGTA,
3mM MgCl₂·6H₂O, 60 mM potassium K-lactobionate, 20 mM taurine, 10 mM KH₂PO₄, 20 mM HEPES, 110 mM Sucrose, 1 g/l fatty acid free BSA).

**High-Resolution Respirometry.** Mitochondria respiratory states were assessed by high-resolution respirometry (Oxygraph O2k, Oroboros Instruments, Innsbruck, Austria) using a standard substrate uncoupler inhibitor titration (SUIT) protocol described previously (6). Mitochondrial oxidative capacity (State 3) was measured using substrates specific for respiratory chain complex I (10mM glutamate, 2mM malate), complex I and II (10mM glutamate, 2mM Malate, 10mM succinate), and complex II alone (10mM glutamate, 2mM malate, 10mM succinate, 0.5 µM rotenone) in the presence of 2.5mM ADP. Datlab software (Oroboros Instruments, Innsbruck, Austria) was used to calculate the oxygen flux rates (\(J_O_2\): pmol/s/ml) after correcting for background oxygen kinetics (6). Subsequently, \(J_O_2\) were normalized in three ways i) per gram muscle wet weight (pmol/s/g tissue), ii) per mg mitochondrial protein (pmol/s/mg protein), and per unit of citrate synthase activity (pmol/s/citrate synthase activity). A few OXPHOS measures were excluded from analysis due to technical issues during the assay as indicated.

**Citrate Synthase Activity.** Citrate synthase activity was measured spectrophotometrically in tissue homogenates and in isolated mitochondria using a commercially available citrate synthase activity kit (Sigma Aldrich).

**Quantitative Real-Time PCR (qPCR).** mRNA abundance was quantified using qPCR as described (7, 8). Gene transcripts for **pgc1 a total**, **pgc1a1**, **pgc1a 4**, **tfam**, **nrf1**, **nrf2**, **sirt1**, **sirt3**, **cox3**, **cox4**, **sdh subunit b**, **atps5f1**, **cytochrome bc complex**, **nd4**, **myolc**, **mhcl**, **mhclla**, and **mhcllx** were quantified using qPCR as previously described (5, 7). In a subset of samples, meteorin like (**Metrnl**) mRNA abundance was measured as previously described (9). The gene transcripts were chosen as they encoded proteins that regulate mitochondrial biogenesis (e.g., transcription factors) as well as encode proteins of the electron transport chain. In addition, we also measured the expression of proteins encoding myosin light chain (**myolc**) as well as myosin heavy chain isoforms (**mhcl**, **mhclla**, **mhcllx**). One subject was excluded from all mRNA analyses due to
being a statistical outlier on multiple gene transcripts. One subject was excluded from 
the pgc1a total and two from the pgc1a1 mRNA analyses due to technical issues.

**Immunoblotting.** Protein abundance of PGC1α, SIRT3, TFAM, and Vinculin were 
quantified using standard immunoblotting techniques as previously described (10) with 
minor modifications in a subset of samples (n=60; n=10 young, 10 old in each 
intervention group, 50:50 male:female). Frozen muscle tissue was pulverized in liquid 
nitrogen to a fine powder, which was subsequently homogenized on ice in an NP40 cell 
lysis buffer (100mM NaCl, 20mM Tris-HCl, 0.5mM EDTA, 0.5% (v/v) Nonidet P40, 
which included HaltPhosphotase and Complete Mini Protease inhibitor cocktails).

Following the homogenization each sample was sonicated at 4°C in a sonication bath 
and subsequently rocked at 4°C for 20 min. Finally, the homogenate was centrifuged at 
14,000 g to remove cell debris, the supernatant was transferred to a fresh Eppendorf 
tube, and the protein concentration of the supernatant was determined using the Pierce 
660nM Protein Assay Kit (Thermo-Fisher Scientific, Rockford, IL). Samples were 
prepared in NuPAGE LDS Sample Buffer (Thermo-Fisher Scientific, Rockford, IL) with 5 
mM tris(2-carboxyethyl)phosphine (TCEP) to final concentration of 3 µg/ul and 
incubated at 70°C for 10 min to denature proteins. A total of 45 µg protein was loaded 
to each well of precast gels (20 lane, 4-12% NuPage Novex Bis-Tris Midi Gel, 
Invitrogen, Carlsbad, CA). Since multiple gels were required to run the complete study, 
each membrane contained a quality control (QC) sample that was used to normalize the 
experimental samples for comparisons across different gels. Proteins were then 
separated at 200 V for ~ 1 h using a XCell4 SureLock Midi-Cell (Invitrogen, Carlsbad, 
CA) and transferred at 20 V for 1 h using a BioRad Semi-Dry Transfer Apparatus 
(Biorad Transblot). The membranes were briefly washed in tris buffered saline (TBS) 
and then allowed to dry. Subsequently, the membranes were blocked for 1 h in LiCor 
Blocking Buffer (Li-Cor, Lincoln NE). The membranes were then incubated overnight at 
4°C with gentle agitation in the following primary antibodies for PGC1α (Abcam), SIRT3 
(Abcam), Vinculin (Abcam), and TFAM (Abcam) and the Mitoscience Total OXPHOS 
Mitochondrial Antibody Cocktail (Abcam) which produces five representative electron 
transport chain bands: band 1 (ATP5A, complex V), band 2 (UQCR2, complex III), band
3 (SDHB, complex II), band 4 (COXII, complex IV), and band 5 (NDUFB8, complex I). The membranes were then washed in TBST (TBS plus tween) and incubated in appropriate anti-mouse and anti-rabbit fluorescent secondary antibodies (DyLight, Thermofisher, Rockford, IL) for 1 h in the dark. The membranes were then washed in TBST with a final wash performed in TBS. Proteins were detected and quantified using the Li-Cor Odyssey fluorescent detector (Li-Cor, Lincoln NE). Note: PGC1α, SIRT3, and Vinculin blots were performed on the same membrane through serial blotting. TFAM and the Mitosciences Total OXPHOS Antibody Cocktails were performed on separate blots. The integrated intensity of bands detected by the Mitosciences Total OXPHOS antibody cocktail ranging from ~10 kDa through ~60 kDa was used to represent the abundance of total OXPHOS ETC proteins. We excluded one older ET participant from the Total OXPHOS ETC analysis due to a smear in their bands.


