Figure EV1. Increased PGC-1α expression in MCK-miR-499 muscle.

A Upstream regulator analysis by Ingenuity Pathways Analysis (IPA) based on the gene expression array data generated from the gastrocnemius muscle of MCK-miR-499. The top five upstream regulators are shown with the predicted z-scores.

B Expression of genes encoding PGC-1α, ERR, and PPAR transcription factors (RT-qPCR) in gastrocnemius muscle compared to NTG controls (n = 5 mice per group).

C Representative Western blot analysis of PGC-1α (Top) and myoglobin (Bottom) in white vastus (WV), gastrocnemius (GC), and soleus (Sol) muscle from the indicated genotypes (n = 4 mice per group).

D Results of qPCR to determine mitochondrial DNA levels in WV muscle of the indicated genotypes using primers for NADH dehydrogenase (Nd1, mitochondria-encoded) and lipoprotein lipase (Lpl, nuclear-encoded). Nd1 levels were normalized to Lpl DNA content and expressed relative to NTG (= 1.0) muscle (n = 5 mice per group). P = 0.569 (NS, not significant).

Data information: All values represent the mean ± SEM. P-value was determined using two-tailed unpaired Student’s t-test. Source data are available online for this figure.
Figure EV2. PGC-1α-independent regulation of the slow-twitch muscle fiber program by miR-499.

A  Representative Western blot analysis of PGC-1α protein expression in the gastrocnemius muscle from the indicated genotypes (n = 4 mice per group).
B  Morphology of dissected gastrocnemius/soleus (asterisk) and tibialis anterior (TA) muscle.
C  Expression of genes encoding slow-twitch/fast-twitch troponin and Sox6 (RT-qPCR) in soleus (Sol), gastrocnemius (GC), and white vastus (WV) muscle from the indicated genotypes. For Sol, NTG, n = 10; 499Tg, n = 9; PGC-1α mKO, n = 8; 499Tg/PGC-1α mKO, n = 10. For GC and WV, n = 5 mice per group. *P < 0.05 (versus NTG), †P < 0.05 (versus 499Tg).

Data information: Values represent the mean (± SEM) and are shown as arbitrary units (AU) normalized (~ 1.0) to the value of the NTG control. P-value was determined using one-way ANOVA coupled to a Fisher’s least-significant difference (LSD) post hoc test.

Source data are available online for this figure.
Figure EV3. Identification of miR-499 targets.

A. Diagram shows the miR-499 target identification. The TargetScan and MicroCosm programs were used to identify putative target mRNAs for miR-499, and this list was cross-matched for genes that were downregulated in MCK-miR-499 muscle (fold change < -1.2). The overlapping putative targets, together with those predicted targets that are known to be involved in the regulation of energy metabolism, were chosen for further UTR luciferase validation assay.

B. 3'UTR luciferase reporters containing the predicted binding site of miR-499 were used in cotransfection studies in HEK293T cells in the presence or absence of plasmids expressing miR-499 (n = 3 independent experiments). Sox6 3'UTR containing the binding site of miR-499 was used as a positive control. *P < 0.01. All values represent the mean ± SEM and are shown as arbitrary units (AU) normalized to corresponding controls. P-value was determined using two-tailed unpaired Student's t-test.
Figure EV4. miR-499 activation reverses the diminished oxidative muscle fiber program in mdx muscle.

A (Left) Representative Western blot analysis performed on extracts of the gastrocnemius muscle isolated from the indicated genotypes using phospho-AMPKα (Thr172) and AMPKα antibodies. (Right) Quantification of the p-AMPKα/AMPKα signal ratios normalized (= 1.0) to the NTG control. WT, n = 6; mdx, n = 6; mdx/499Tg, n = 5. *P < 0.0001 (versus WT), ‡P < 0.0001 (versus mdx).

B Expression of the Ldhb and Ldha genes (RT-qPCR) in muscle from the indicated genotypes (n = 5 mice per group). Ldhb: *P = 0.0006 (versus WT), ‡P < 0.0001 (versus mdx); Ldha: *P = 0.059 (versus WT), ‡P = 0.0052 (versus mdx).

C A representative LDH isoenzyme activity gel is shown (n = 3 mice per group).

D Mitochondrial respiration rates were determined from the extensor digital longus muscle of the indicated genotypes using pyruvate/malate as substrate. Pyruvate/malate (P/M)-stimulated, ADP-dependent respiration, and oligomycin-induced (oligo) are shown. mdx, n = 6; mdx/499Tg, n = 6. *P = 0.0474 (ADP).

E Results of qPCR to determine mitochondrial DNA levels in WV muscle of the indicated genotypes using primers for NADH dehydrogenase (Ndi1, mitochondria-encoded) and lipoprotein lipase (Lpl, nuclear-encoded). mdx, n = 5; mdx/499Tg, n = 6. P = 0.0989 (NS, not significant).

Data information: All values represent the mean ± SEM. P-value in (A and B) was determined using one-way ANOVA coupled to a Fisher’s least-significant difference (LSD) post hoc test; P-value in (D and E) was determined using two-tailed unpaired Student’s t-test.

Source data are available online for this figure.
Figure EV5. Restoring the expression of miR-499 reduces muscle damage in mdx mice.

A Bar histograms represent size distribution of the muscle fibers from the indicated genotypes. Relative fiber size was quantified using Image-Pro Plus software (n = 5 mice per group).

B Quantification of muscle fibers with centrally located nuclei in indicated genotypes. Values represent the mean % (± SEM) total muscle fibers from n = 5 mice per group and five images per muscle. *P = 0.0306 (versus mdx).

C Representative images of Evans blue dye infiltration in damaged myofibers of the tibialis anterior (TA) muscle from indicated genotypes. Scale bar: 500 μm.

Data information: Values represent the mean ± SEM. P-value was determined using two-tailed unpaired Student’s t-test.