Resveratrol Ameliorates the Muscular Pathology in the Dystrophic Mdx Mouse, a Model for Duchenne Muscular Dystrophy

Yusuke S. Hori, Atsushi Kuno, Ryusuke Hosoda, Masaya Tanno, Tetsuji Miura, Kazuaki Shimamoto and Yoshiyuki Horio

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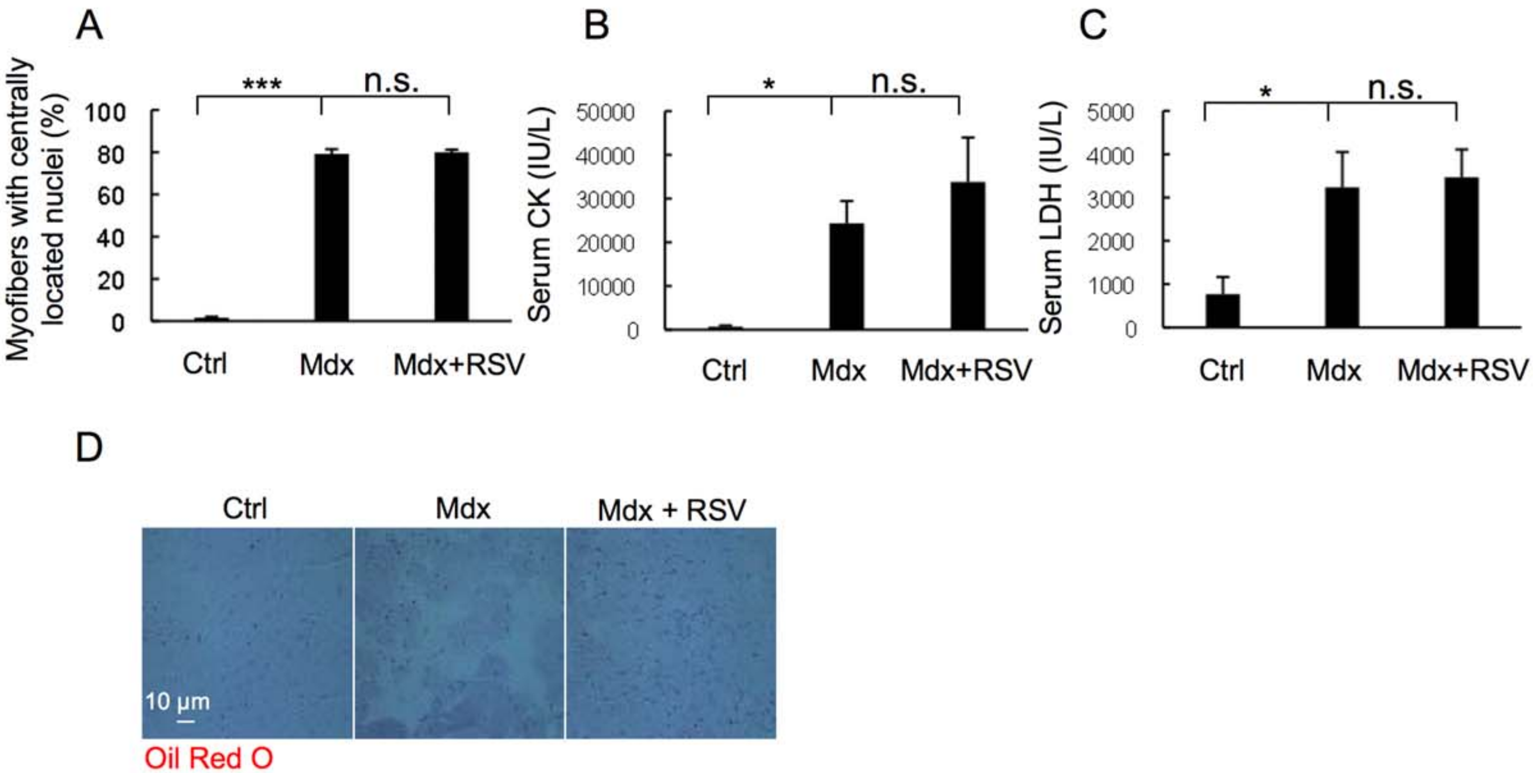

Supplemental Figure 1. Number of myofibers with central nuclei and serum activities of CK and LDH. (A) Sections of biceps femoris were stained with phalloidin and Hoechst 33342 as shown in Fig. 1A and the number of myofibers with and without centrally located nuclei per an image was counted and compared among control (Ctrl), untreated mdx (Mdx) and resveratrol-treated mdx mice (Mdx + RSV). Eight independent sections of biceps femoris from a mouse were examined and data from three mice in each group were compared. (B, C) Activities of serum creatine kinase (CK) and lactate dehydrogenase (LDH) in control (Ctrl), untreated mdx (Mdx) and resveratrol-treated mdx mice (Mdx + RSV) (n = 5-6 mice /each group). (D) Fat deposition was not detected in muscles from mdx mice. Representative sections stained by Oil Red O.

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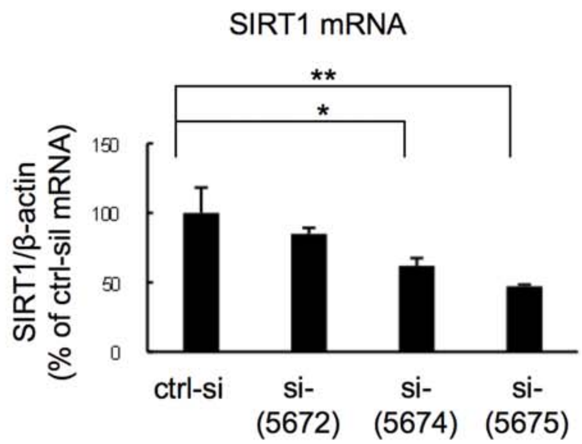

Supplemental Figure 2. Effect of SIRT1 siRNAs on SIRT1 mRNA expression levels. Effects of SIRT1 siRNAs and control siRNA (ctrl-si) on SIRT1 mRNA levels in C2C12 cells were assessed by qRT-PCR (n = 3). SIRT1-si(5675) was the most effective siRNA among three SIRT1 siRNAs. **P<0.01 and *P<0.05.

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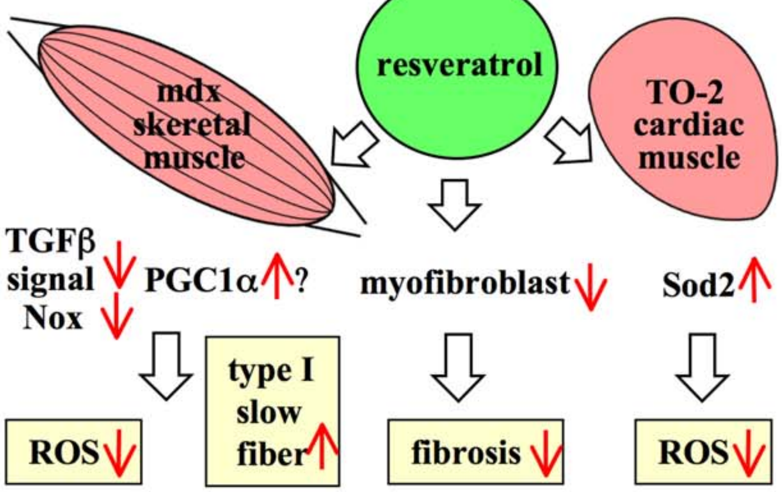

Supplemental Figure 3. Functions of resveratrol on skeretal myocytes, myofibroblasts and cardiomyocytes. Resveratrol did not reduce TGF-β1 level but seems to inhibit TGF-β signal and activates PGC1α, resulting in reduced ROS levels and the increase of type I fibers in the biceps femoris of mdx mice. Resveratrol also suppressed the expression of the Nox4, Duox1, and p47 ^{phox} mRNAs. On the other hand, resveratrol induced Sod2 in the failing hearts of TO-2 hamsters (Tanno et al., 2010). Resveratrol inhibited the progression of fibrotic events in the skeletal muscle of mdx mice and the cardiac muscle of TO-2 hamsters (Tanno et al., 2010).

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Supplemental Table 1 Primers used in experiments.

	Forward	Reverse
MHC1	5'-CCAAGGGCCTGAATGAGGAG-3'	5'-GCAAAGGCTCCAGGTCTGAG-3'
MHC2b	5'-ACAAGCTGCGGGTGAAGAGC-3'	5'-CAGGACAGTGACAAAGAACG -3'
Slow troponin	5'-TGCCGGAAGTTGAGAGGAAATCCAAGAT-3'	5'-CCAGCACCTTCAGCTTCAGGTCCTTGAT-3'
Fast troponin	5'-AAATGTTCGAGTCTGAGTCCTAACTG-3'	5'-GCCAAGTACTCCCAGACTGGAT-3'
Collagen Ia1	5'-ATGTTCAGCTTTGTGGACCT-3'	5'-CAGCTGACTTCAGGGATGT-3'
Collagen Ia2	5'-TGTTGGCCCATCTGGTAAAGA-3'	5'-CAGGGAATCCGATGTTGCC-3'
Fibronectin	5'- GCGACTCTGACTGGCCTTAC-3'	5'-CCGTGTAAGGGTCAAAGCAT-3'
CD45	5'-ATCCCATTGAAAGTGGATTCAGTT-3'	5'-AGCACTCTTACATACGCTTATCTATATC-3'
TNF-α	5'-GACCCTCACACTCAGATCATCTTCT-3'	5'-CCTCCACTTGGTGGTTTGCT-3'
IL-1β	5'-TCGTGCTGTCGGACCCATAT-3'	5'-GTCGTTGCTTGGTTCTCCTTGT-3'
TGF-β1	5'-TGGAAAGGGCCCAGCAC-3'	5'-GCAATAGTTGGTATCCAGGGCT-3'
TGF-β2	5'-CTTCGACGTGACAGACGCT -3'	5'-GCAGGGGCAGTGTAAACTTATT-3'
TGF-β3	5'-CAGGCCAGGGCAGTCAGAG-3'	5'-ATTTCCAGCCTAGATCCTGCC-3'
Nox1	5'-TAAATTGCCCCTCCATTTCC-3'	5'-CCCTGCTGCTCGAATATGAA-3'
Nox2	5'-CCCACCCTTTCAAAACCATT-3'	5'-ACTTGGATACCTTGGGGCAC-3'
Nox4	5'-CGAGACTTTTCATTGGGCGTCCTC-3'	5'-TAGAACTGGGTCCACAGCAGAAAA-3'
Duox1	5'-GTTCACTGAGGCACACCGAG-3'	5'-GTGGCGCCTGTAATTCTCAA-3'
Duox2	5'-ACCACGACAGTGATCTCCGA-3'	5'-CGACAGGCACTGCTTTGTTT-3'
p22phox	5'-GGGAAAGAGGAAAAAGGGGT-3'	5'-AGTAATTCCTGGTGAGGGGC-3'
p47phox	5'-GAGGTTGGGTCCCTGCAT-3'	5'-GCTTTGATGGTTACATACGGTTC-3'
Sod1	5'-CAGGACCTCATTTTAATCCTCAC-3'	5'-TGCCCAGGTCTCCAACAT-3'
Sod3	5'-GGGGAGGCAACTCAGAGG-3'	5'-TGGCTGAGGTTCTCTGCAC-3'
Hmox1	5'-GTCAAGCACAGGGTGACAGA-3'	5'-CTGCAGCTCCTCAAACAGCT-3'
Nqo1	5'-ACATGAACGTCATTCTCTGG-3'	5'-ACCAGTTGAGGTTCTAAGAC-3'
Gclm	5'-TGTGTGATGCCACCAGATTTG-3'	5'-ATGCTTTCTTGAAGAGCTTCCT-3'
Gclc	5'-ATTGTCGCTGGGGAGTGATT-3'	5'-TATCTATTGAGTCATACCGAGA-3'
SIRT3	5'-GCTGCTTCTGCGGCTCTATAC-3'	5'-GAAGGACCTTCGACAGACCGT-3'