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Supplemental Material to:

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PARK2/Parkin-mediated mitochondrial clearance contributes to proteasome activation during slow-twitch muscle atrophy via NFE2L1 nuclear translocation

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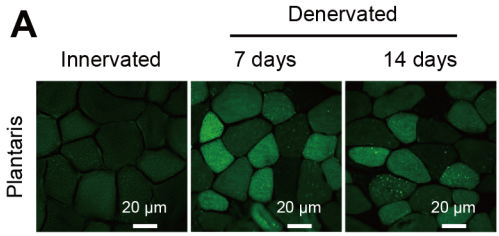
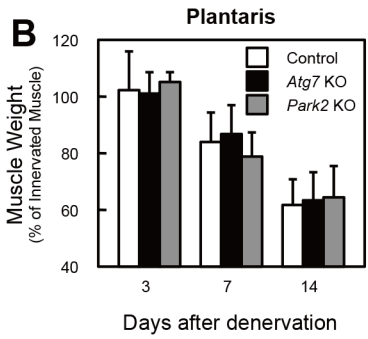
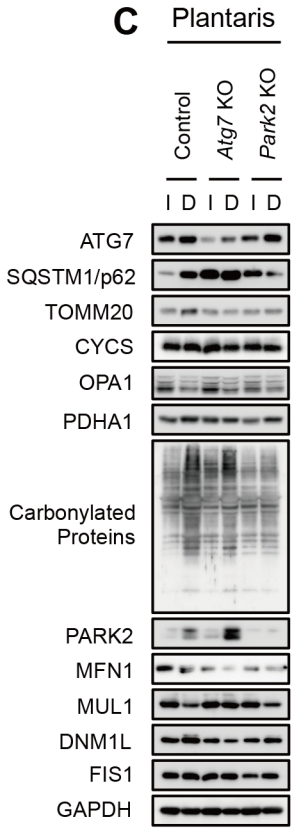
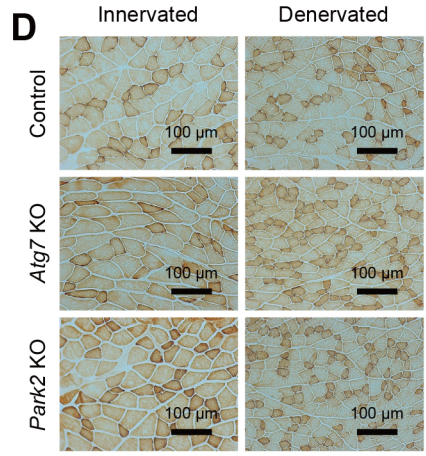
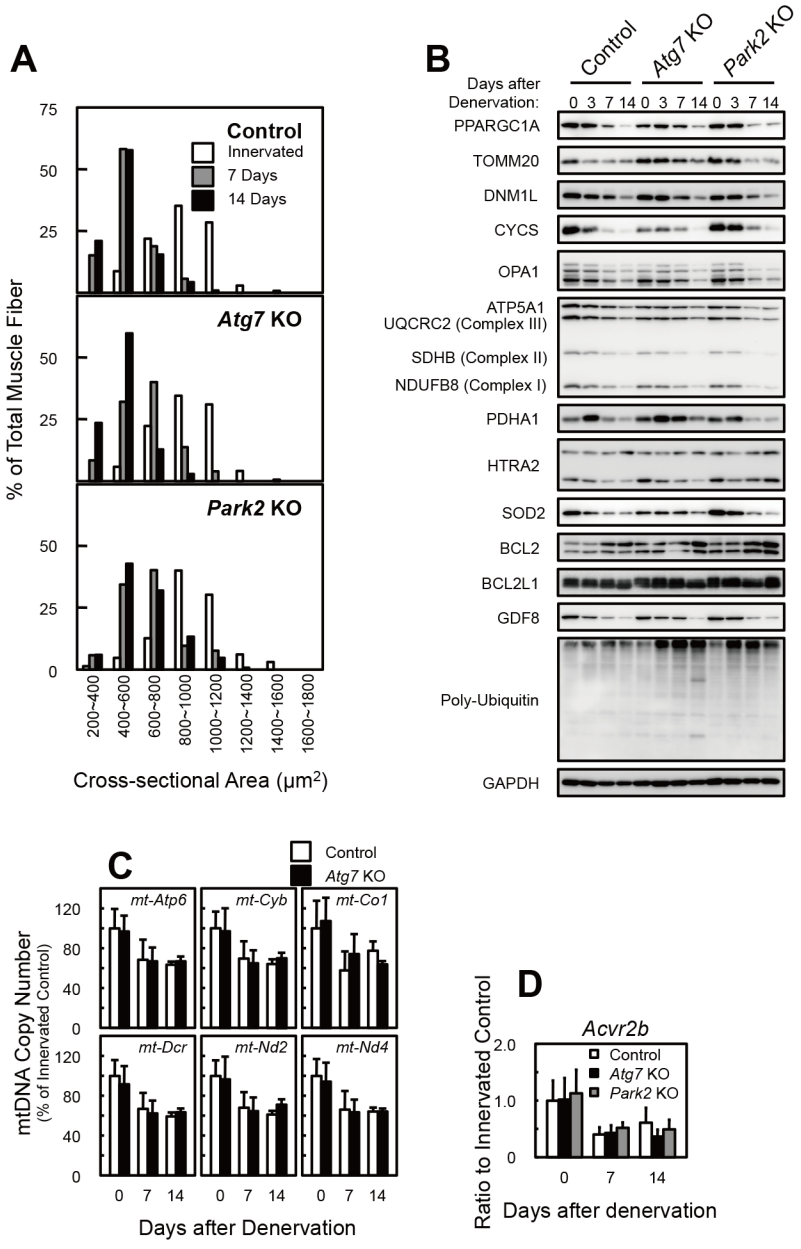




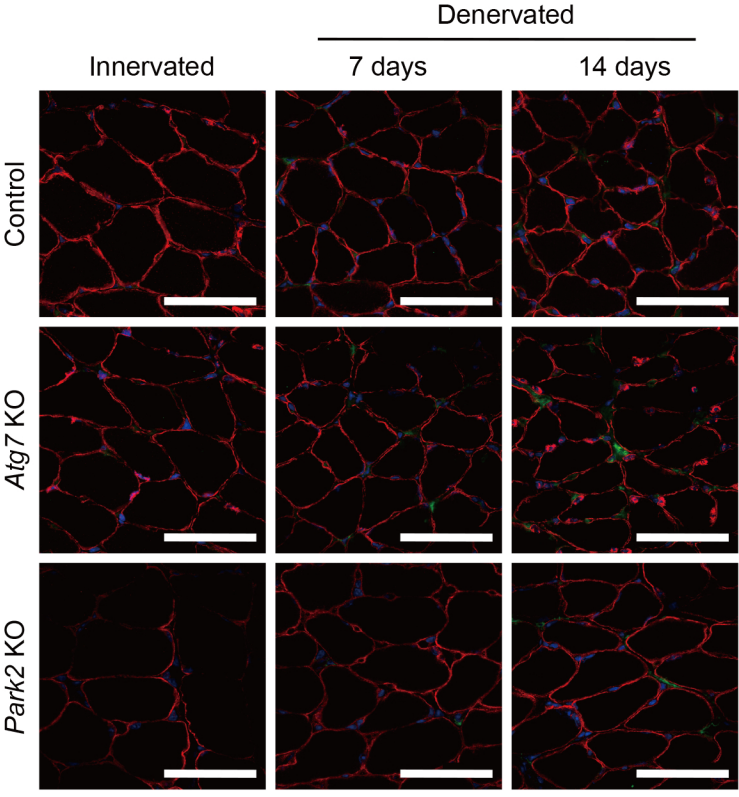




Furuya et al. Supplementary Fig. S1



Furuya et al. Supplementary Fig. S2



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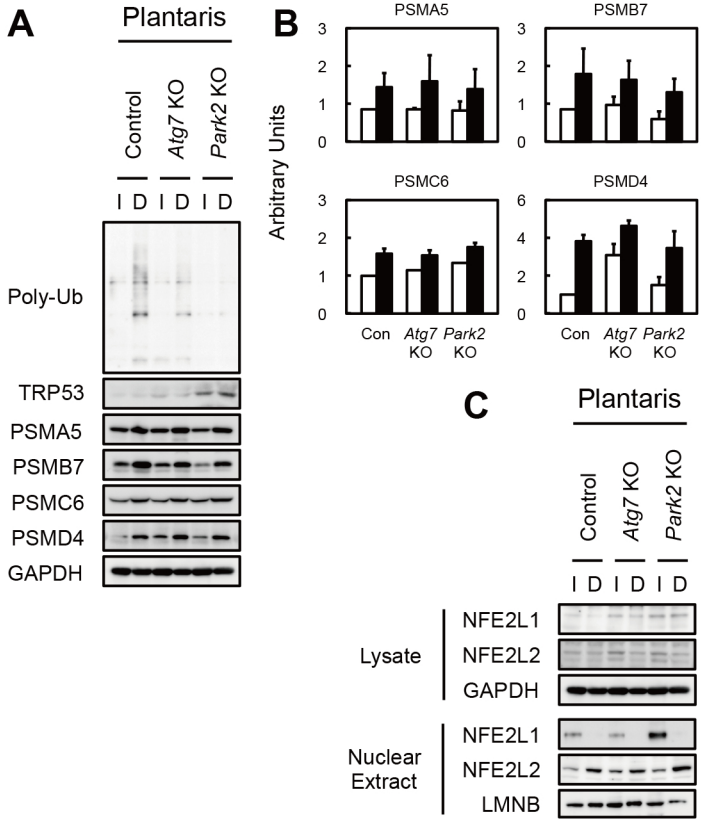

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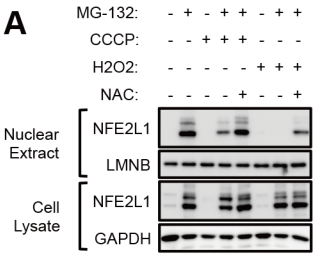
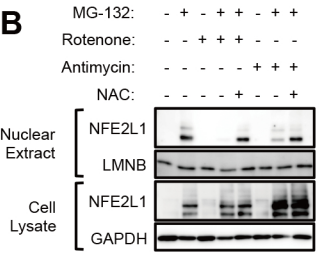
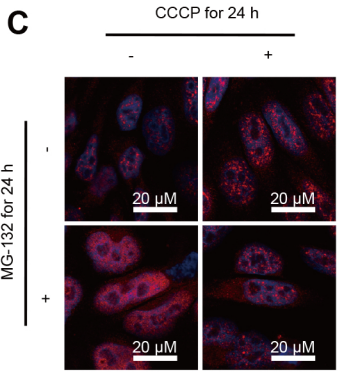
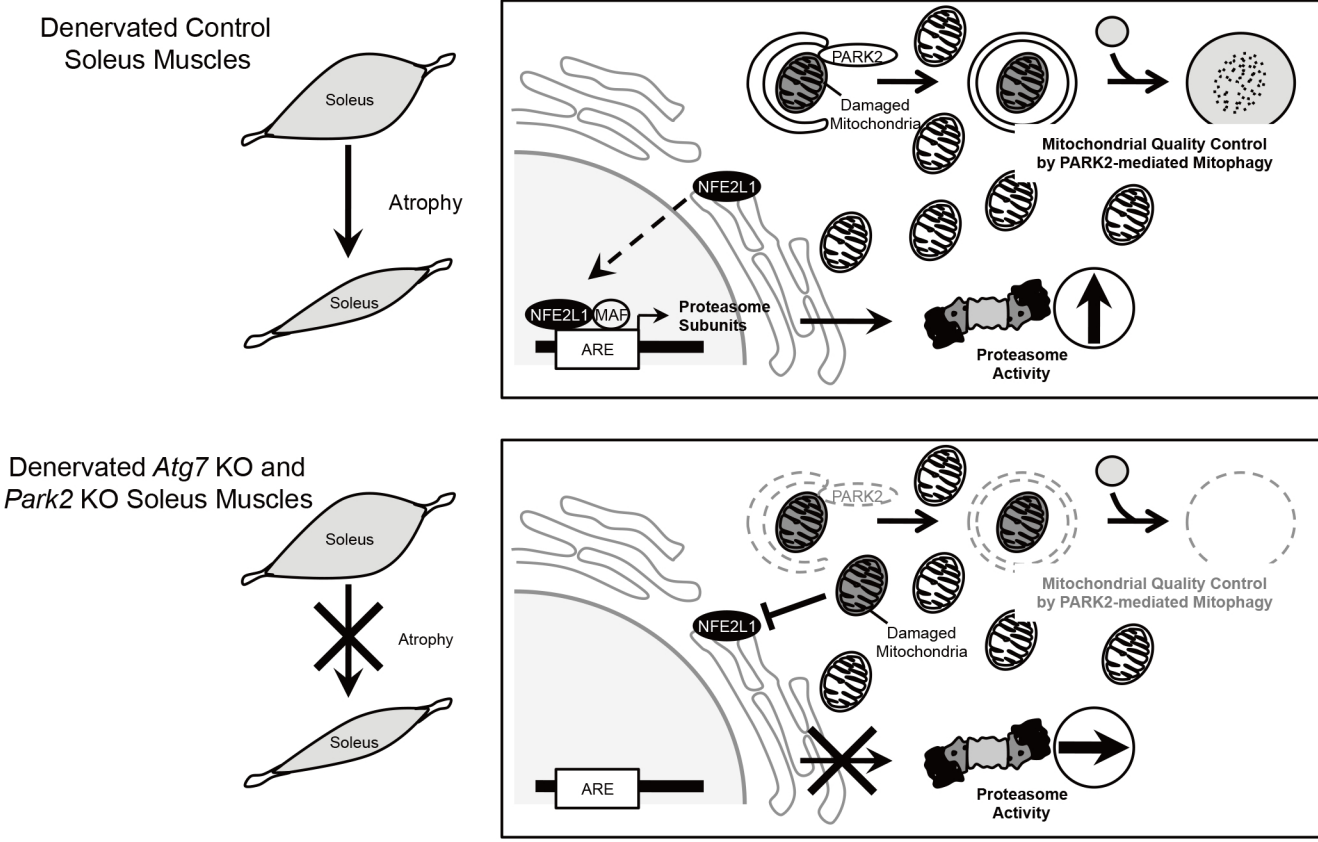






Furuya et. al. Supplementary Figure S5.



Furuya et al. Supplementary Fig. S6

Supplementary Figure Legends

Figure S1. Effect of denervation on fast-twitch plantaris muscles. (**A**) Representative images of plantaris muscles from GFP-LC3 transgenic mice at 0 (innervated), 7 and 14 days after denervation. Scale bar, 20 μm. (**B**) Cutting of sciatic nerves was performed. Denervated plantaris muscle weights are shown as a percentage of the contralateral innervated muscle weights. Data are shown as means ± s.d. (**C**) Western blot analysis of plantaris muscles from mice at 7 days after denervation. Whole tissue lysates of the denervated (D) and the contralateral innervated (I) plantaris muscles were immunoblotted with antibodies against the indicated proteins. (**D**) Histochemical detection of cytochrome *c* oxidase (Cox) activities in cryosections of plantaris muscles from control, *Atg7* KO and *Park2* KO mice 7 days after denervation. Scale bars, 100 μm.

Figure S2. Time course of changes in muscle and the levels of mitochondrial markers, myostatin signalling pathway components, and members of the anti-apoptotic BCL2 family in soleus muscles during denervation atrophy. (**A**) Cross-sectional areas of soleus

muscle fibres from denervated mice at seven days (grey bars) or 14 days (closed bars) post-denervation or innervated mice (open bars) were quantified using imageJ software. (**B**) Whole tissue lysates of soleus muscles at 0, 3, 7 and 14 days after denervation were immunoblotted with antibodies against the indicated proteins. The data shown are representative of at least 3 separate experiments. (**C**) The time course of the changes in mtDNA copy numbers in denervated soleus muscles from *Atg7* KO and control mice. mtDNA copy numbers were quantified by real-time PCR to detect mtDNA-encoded genes. Data are shown as the ratios (mean ± s.d.) to the mRNA levels obtained from innervated soleus muscles from control mice. (**D**) *Acvr2b* (myostatin receptor) mRNA levels in soleus muscles were quantified by real-time PCR. Data are shown as the ratios (mean ± s.d.) to the mRNA levels obtained from innervated soleus muscles from control mice.

Figure S3. Accumulation of reactive oxygen species in the soleus muscles of denervated *Atg7* KO and *Park2* KO mice. Immunofluorescence images of denervated soleus muscles stained with anti-8-OHdG (an oxidative stress marker, green) and

anti-DMD (red) antibodies. Nuclei were visualized with Hoechst 33342 (blue). Bar, 20 μm.

Figure S4. Denervation induces the expression of proteasome subunits in plantaris muscles. (**A**) Total tissue lysates of denervated and innervated plantaris muscles were immunoblotted using antibodies against the indicated proteins. (**B**) The levels of mRNAs for proteasome subunits in plantaris muscles were quantified by real-time PCR. (**C**) Nuclear levels of Nrfs in plantaris muscles. Nuclear extracts and total lysates of plantaris muscles were immunoblotted with anti-NFE2L1, anti-NFE2L2, anti-LMNB and anti-GAPDH antibodies.

Figure S5. Effects of mitochondrial inhibitors on NFE2L1 nuclear translocation. (**A**,**B**) NFE2L1 levels in the cell lysates and nuclear extracts of C2C12 cells incubated with 10 μM CCCP, 250 μM H_2O_2 and/or 10 μM MG-132 (**A**), or with 5 μg/ml rotenone, 5 μM antimycin and/or 10 μM MG-132 (**B**) in the presence or absence of 10 mM NAC, for 24 h were assayed by western blotting with anti-NFE2L1, anti-LMNB (as a loading control

for nuclear extracts) and anti-GAPDH (as a loading control for cell lysates) antibodies. (**C**) HeLa cells incubated with MG-132 and/or CCCP for 24 h were immunostained with anti-NFE2L1 antibody (red). Nuclei were visualized with Hoechst 33342 (blue).

Figure S6. Scheme of the contribution of PARK2-mediated mitophagy to soleus muscle atrophy. In soleus muscle from *Atg7* KO and *Park2* KO mice, owing to a lack of PARK2-mediated mitophagy, damaged mitochondria are accumulated following denervation. ROS produced by damaged mitochondria interfere with the activation of the transcription factor NFE2L1, which regulates expression of proteasome subunits. Consequently, a delay in soleus muscle atrophy occurs. Conversely, the increase in the number of proteasomes following NFE2L1 activation causes atrophy of the soleus muscle from denervated wild-type animals, because of the maintenance of mitochondrial quality by PARK2-mediated mitophagy.

Table S1

The following primers were used for quantitative RT-PCR experiments.

Gene	Forward Primer	Reverse Primer
Park2	AAACCGGATGAGTGGTGAGT	AGCTACCGACGTGTCCTTGT
Fbxo32	CACATTCTCTCCTGGAAGGGC	TTGATAAAGTCTTGAGGGGAAAGTG
Trim63	TGGTGGAAAACATCATTGACATC	GTTGATCTTCTCGTCTTCGTGTTC
Acvr2b	ACTGGGAGCTGGAGCGCACCAAC	GAAGTTGCCTTCGCAGCAGCAGAA
Psma7	AACGTCTGTATGGCCTTTGC	GTCACTGGGTCCTCCACTGT
Psmb4	TTCACTGGCCACTGGTTATG	CGAACGGGCATCTCTGTAGT
Pamc4	TGGTCA TCGGTCAGTTCTTG	CGGTCGATGGTACTCAGGAT
Psmd1	GGGGCTTTTGAGGAGTCTCT	GCAAA TCTGCA TTTTCCACA
Gapdh	CACCATCTTCCAGGAGCGAG	CCTTCTCCATGGTGGTGAAGAC

Table S2

The following primers were used for quantification of mtDNA copy numbers

Gene	Forward Primer	Reverse Primer
mt-Co1	ACTATACTACTACTAACAGACCG	GGTTCTTTTTTTCCGGAGTA
mt-Nd2	CACGATCAACTGAAGCAGCAA	ACGATGGCCAGGAGGATAATT
mt-Nd4	ATTATTATTACCCGATGAGGGAACC	ATTAAGATGAGGGCAATTAGCAGT
mt-Cyb	GCCACCTTGACCCGATTCT	TTGCTAGGGCCGCGATAAT
mt-Atp6	AATTACAGGCTTCCGACACAAAC	TGGAATTAGTGAAATTGGAGTTCC
mt-Dcr	AATCTACCATCCTCCGTGAAACC	GCCCGGAGCGAGAAGAG
Ppia	ACACGCCATAATGGCACTGG	CAGTCTTGGCAGTGCAGAT