Supplementary Data

Exercise Restores Muscle Stem Cell Mobilization and Regenerative Capacity and muscle metabolic alterations via Adiponectin/AdipoR1 Activation in SAMP10 mice

Aiko Inoue, Xian Wu Cheng, Zhe Huang, Lina Hu, Ryosuke Kikuchi, Haiying Jiang, Limei Piao, Takeshi Sasaki, Kohji Itakura, Hongxian Wu, Guangxian Zhao, Yanna Lei, Guang Yang, Enbo Zhu, Xiang Li, Kohji Sato, Teruhiko Koike, Masafumi Kuzuya

Corresponding Author: Dr. Xian Wu Cheng

Department of Community Health & Geriatrics, Nagoya University, Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8550, Japan

Tel: +81-52-744-2364; Fax: +81-52-744-2371

E-mail: xianwu@med.nagoya-u.ac.jp

Suppl. Table S1. Primer sequences used in the quantitative real-time polymerase chain reaction (PCR)

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	GenBank no.
Adiponectin	AGGGAGAGAAAGGAGATGCAG	CTTTCCTGCCAGGGGTTC	NM_009605
AdipoR1	CGGTGTTGACGAGGCGTCCGAAG	GGTCTTCGGGATGTTCTTCCTG	NC_000067.6
COX-III	TCTTCATGGCTACTGGATTCCA	ATCATGCTGCGGCTTCAAAT	NC_005089
COX-IV	AGCTGAGCCAAGCAGAGAAG	AATCACCAGAGCCGTGAATC	NM_053091
GLUT-4	GACGGACACTCCATCTGTTG	GCCACGATGGAGACATAGC	NM_009204
PGC-1α	CCGAGAATTCATGGAGCAAT	TTTCTGTGGGTTTGGTGTGA	NM_008904
PGC-1β	ACGGTTTTATCACCTTCCGG	ATAGCTCAGGTGGAAGGAGGG	NM_133249
Atrogin-1	ATGCACACTGGTGCAGAGAG	TGTAAGCACACAGGCAGGTC	AF441120
MuRF-1	TGAGGTGCCTACTTGCTCCT	TCACCTGGTGGCTATTCTCC	NC_000070.6
GAPDH	ATGTGTCCGTCGTGGATCTGA	ATGCCTGCTTCACCACCTTCT	NM_008084

AdipoR1, adiponectin receptor1; MURF, muscle RING-finger protein-1; COX-III: cytochrome *c* oxidase-III; COX-IV; GLUT-4, glucose transporter-4; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α.

Suppl. Table S2. Levels of plasma lipid, targeted growth factors, cytokines and others in two experimental groups at indicated time points

Parameter (at 40 wks)	Control (Non-ET)	mAb-adipo (ET)
T-cho (mg/dL)	83.3 ± 3.5	81.6 ± 7.2
LDL (mg/dL)	9.33 ± 0.8	8.00 ± 1.3
HDL (mg/dL)	43.3 ± 3.0	42.4 ± 3.2
Triglyceride	149.3 ± 23.5	128.8 ± 29.7
Glucose (mg/dL)	172.0 ± 17.9	156.0 ± 14.1
BUN (mg/dL)	37.5 ± 1.7	35.0 ± 2.3
Cre (mg/dL)	0.21 ± 0.01	0.21 ± 0.01
Blood examination (32 wks)		
WBC, 10^9/l	0.61 ± 0.10	1.41 ± 0.26*
MON, 10^9/l	0.03 ± 0.01	0.09 ± 0.03
NEU, 10^9/l	0.36 ± 0.07	0.73 ± 0.19
MON, %	8.08 ± 1.89	7.03 ± 1.23
NEU, %	66.2 ± 5.62	43.7 ± 4.21*
RBC, 10^{12}/l	9.39 ± 0.26	9.66 ± 0.13
HGB, g/dL	12.8 ± 0.37	12.7 ± 0.14
HCT, %	45.0 ± 1.26	45.1 ± 1.34
MCH, pg	13.5 ± 0.09	14.0 ± 0.58
MCHC, g/dL	28.4 ± 0.16	28.8 ± 1.08
PLT, 10^9/l	443 ± 12.7	410 ± 31.9-

Values are mean ± SEM. TNF-α, tumor necrosis-factor-α; VEGF, vascular endothelial growth factor; GDF-11, growth differentiation factor; T-cho, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; BUN, blood urea nitrogen; Cre, creatinine; WBC, white blood cells, MON, monocyte; NEU, neutrophils; RBC, red blood cells; HGB, hemoglobin concentration of blood; HCT, hematocrit; MCH, mean corpuscular hemoglobin**;** MCHC, mean corpuscular hemoglobin concentration. *P<0.05, **P<0.01 vs. control.

Suppl. Table S3. Levels of targeted genes in two experimental groups at 32 wks

Parameter (by RT-PCR)	Control (Non-ET)	mAb-adipo (ET)
Soleus muscle	2.90 ± 1.14	0.13 ± 0.05**
COX4	2.90 ± 1.14	0.13 ± 0.05**
COX3	1.24 ± 0.08	1.21 ± 0.07
GLUT4	7.00 ± 2.61	0.74 ± 0.05*
PGC-1α	3.12 ± 1.05	1.03 ± 0.16*
PGC-1β	1.59 ± 0.56	0.33 ± 0.06*
Gastrocnemius		
COX4	1.59 ± 0.47	0.76 ± 0.14**
COX3	0.93 ± 0.12	0.88 ± 0.13
GLUT4	3.37 ± 0.99	0.26 ± 0.12*
PGC-1α	0.81 ± 0.18	0.04 ± 0.04*
PGC-1β	1.98 ± 0.72	0.38 ± 0.13*

Values are mean ± SEM. *P<0.05, **P<0.01 vs. control. Abbreviations are explained in Suppl. Table S1.

Suppl. Figure Legends

Supp. Fig. 1. Schematic representation of the exercise training (ET) program.

Suppl. Fig. 2. Effects of ET on metabolic parameters. **A–D**: Food intake, drinking water, urine volume and excrement weight were recorded in the control (Cont) and exercise training (ET) groups at the indicated time points. Data are mean ± SEM (n=6).

Suppl. Fig. 3. The effects of ET on muscle function and mass at 4 mos post-ET. **A–C**: Body weight (BW), grip strength/BW and endurance were recorded in the control (Cont) and exercise training (ET) groups. **D,E**: The ratios of soleus muscle to BW and ratios of gastrocnemius to BW were calculated at 40 wks of age in both groups. Data are mean ± SEM (n=10–12). NS: not significant.

Suppl. Fig. 4. The effects of ET on the myofiber size and the slow MHC rate at 4 mos post-ET. **A**: Representative H&E staining of soleus and gastrocnemius of Cont and ET mice. **B**: Quantitative data showing the myofiber size in both muscles. **C**: Representative MHC staining images used to assess the content of MHC^+ myofibers in the soleus and gastrocnemius of both groups. **D**: Quantitative data showing the ratios of the MHC^+ myofibers to total myofibers in both muscles. Data are mean ± SEM (n=10–12). Scale bars: 50 μm.

Suppl. Fig. 5. ET ameliorated the mitochondria damage and lipid droplet accumulation in the soleus and gastrocnemius at 4 mos post-ET. **A**: Representative electron microscopy

images show a relatively preserved mitochondrial configuration as well as a small amount of lipid droplets. **B**–**D**: Quantitative data of damaged mitochondrial numbers, percentage of damaged mitochondria. and lipid droplet numbers. Data are mean ± SEM (n=4–6). Scale bar: 500 nm.

Suppl. Fig. 6. Administration of neutralizing pAb against adiponectin (pAb-Adip) suppressed muscle proliferation in 32-wk-old mice. **A**: Representative PCNA immunostaining with the mouse mAb used to assess the content of proliferated cells in both groups. **B**: Quantitative data for PCNA-positive cells. Data are mean ± SEM (n=8–9). Arrowheads: related positive-staining cells. Scale bar: 50 μm.

Suppl. Fig. 7. Effects of adiponectin depletion on intracellular signal molecule changes in the muscles at 2 mos of treatment. **A,B**: Representative Western blot images and quantitative data for the changes of p-AMPKα, p-Akt, p-mTOR, and Bcl-xL proteins in the muscle of both groups. Data are mean ± SEM (n=3).

Suppl. Fig. 8. Adiponectin blocking diminished the ET-mediated amelioration of mitochondria damage and lipid droplet accumulation in the soleus muscles and gastrocnemius at 2 mos of treatment. Representative electron microscopy shows a relatively preserved mitochondrial configuration as well as a small amount of lipid droplets.

Suppl. Fig. 9. Recombinant mouse adiponectin enhanced the levels of of p-AMPKα,

p-mTOR, and Bcl-2 proteins the BM-derived integrin-$\alpha7^+$ stem cells. Representative Western blot images and quantitative data for the changes of p-AMPKα, p-mTOR, and Bcl-2 proteins in a dose-dependent manner. Data are mean ± SEM (n=3).

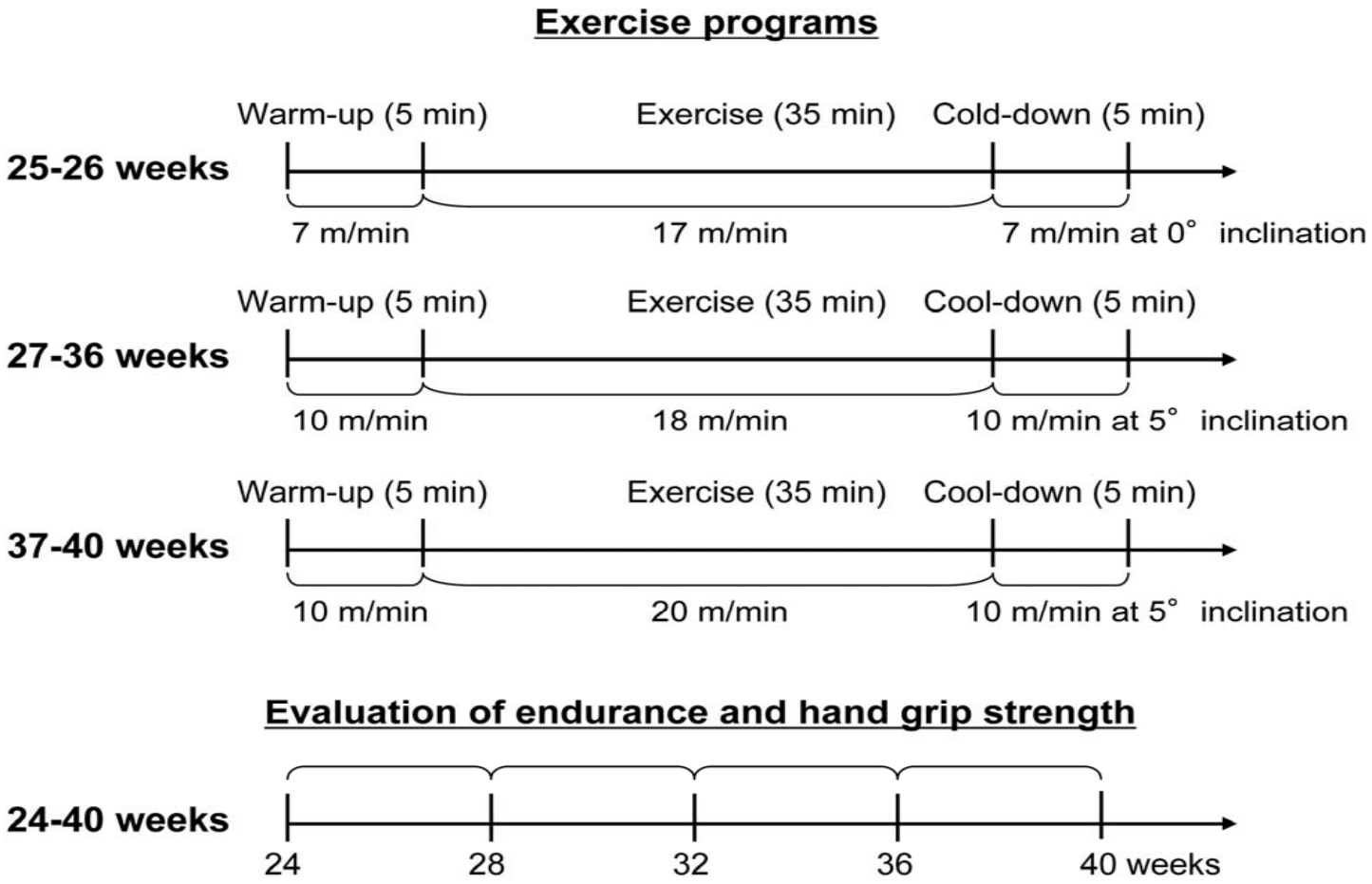
Suppl. Fig. 10. AdipoR1 inhibition abrogated beneficial consequences of exercise on the muscle of SMAP10 mice (24-wk-old). **A,B**: Grip strength/BW and endurance were recorded in exercised mice treated mouse control IgG (Cont group; 450 μg/kg, one/week) or AdipoR1 pAb (AdipoR1-I group; 450 μg/kg, one/week), respectively, for 8 weeks. **C,D**: The ratios of soleus muscle to BW and ratios of gastrocnemius to BW were calculated at 32 wks of age in both groups. **E**: Qauntitative data from double immunofluorescence show the numbers of $CD34^+$/integrin-α_7^+ in soleus muscles and gastrocnemius. **F**: At 2 mos post-ET, $CD34^+$/integrin-α_7^+ was measured by flow cytometry in the BM and PB of both experimental groups. **G**: Representative PCNA immunostaining and combined quantitative data show the content of proliferated cells in the soleus muscles and gastrocnemius of the two experimental groups. **H**: Representative Western blot images and quantitative data show the changes of p-AMPKα, p-mTOR, and Bcl-XL proteins in the muscle of both groups. Data are mean ± SEM (n=3 for Western blots, n=5-6 for others).

Suppl. Fig. 11. AMPK inhibition abrogated beneficial consequences of exercise on aged muscle (24-wk-old). **A–C**: BW, endurance, and drop-out were recorded in exercised mice treated with DMSO (Cont group; 50 μl/day, twice/week) or AMPK inhibitor compound C (AMPK-I group; 10 mg/kg/50μl, twice/week), respectively, for 8 weeks. **D,E**: The ratios

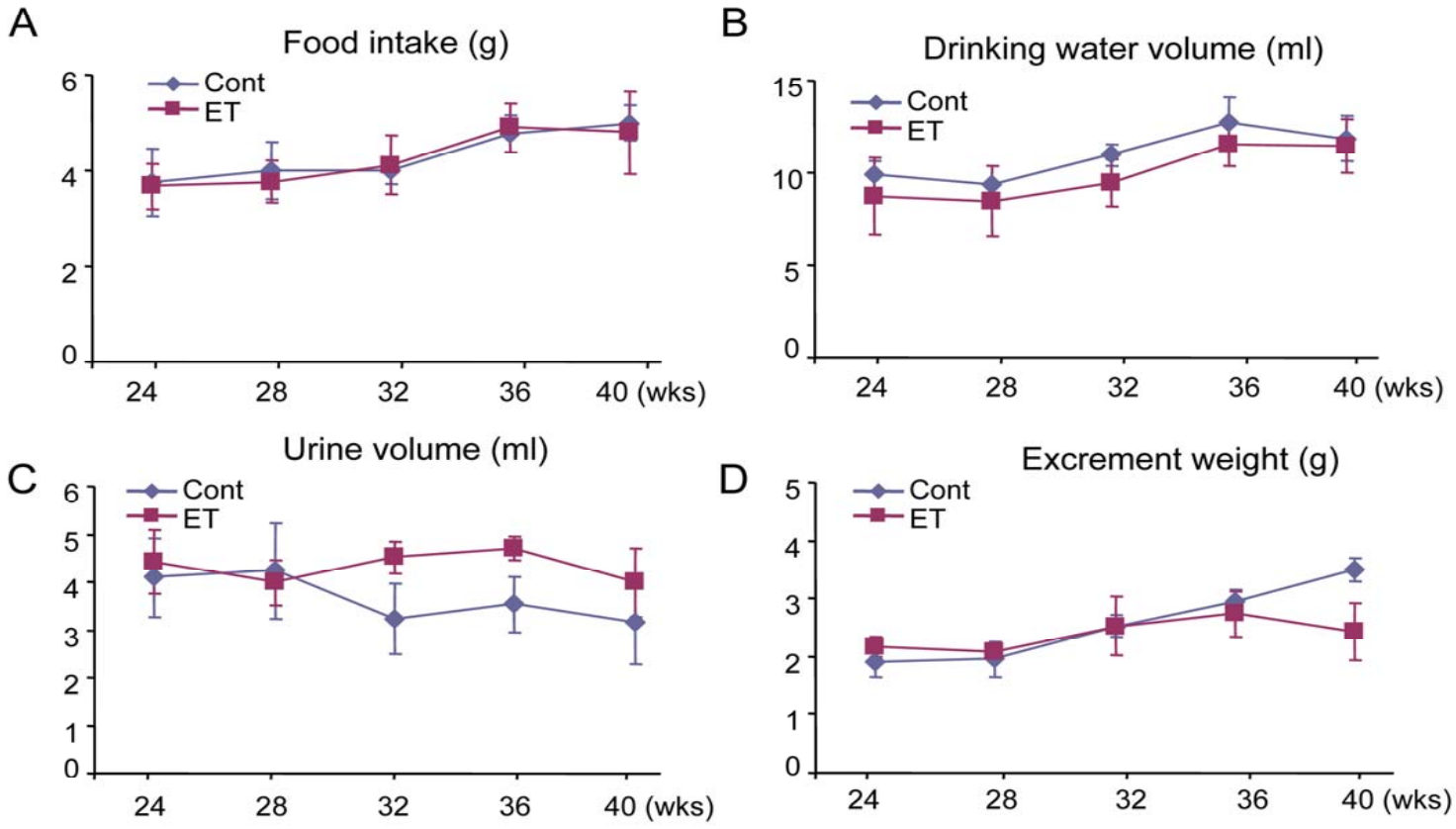
of soleus muscle to BW and ratios of gastrocnemius to BW were calculated at 32 wks of age in both groups. **F**: Representative TUNEL images and quantitative data showing the numbers of apoptotic cells in both muscles of the two groups. White arrowheads indicate TUNEL-positive cells. Data are mean ± SEM (n=5–6). Scar bar, 50 μm.

Suppl. Fig. 12. Proposed mechanism of ET-mediated alleviation of muscle regeneration and dysfunction in a SMAP10 mouse model. MuSCs, muscle stem cells; AMPK, AMP-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; mTOR, mammalian target of rapamycin.

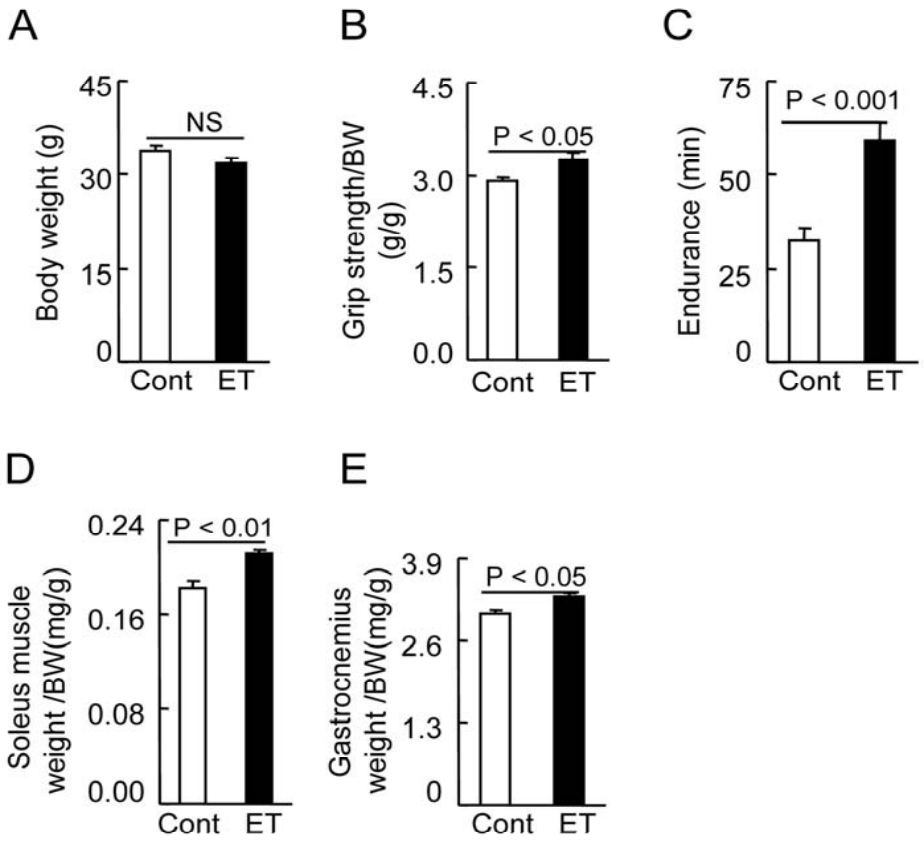
Supplemental Fig. S1



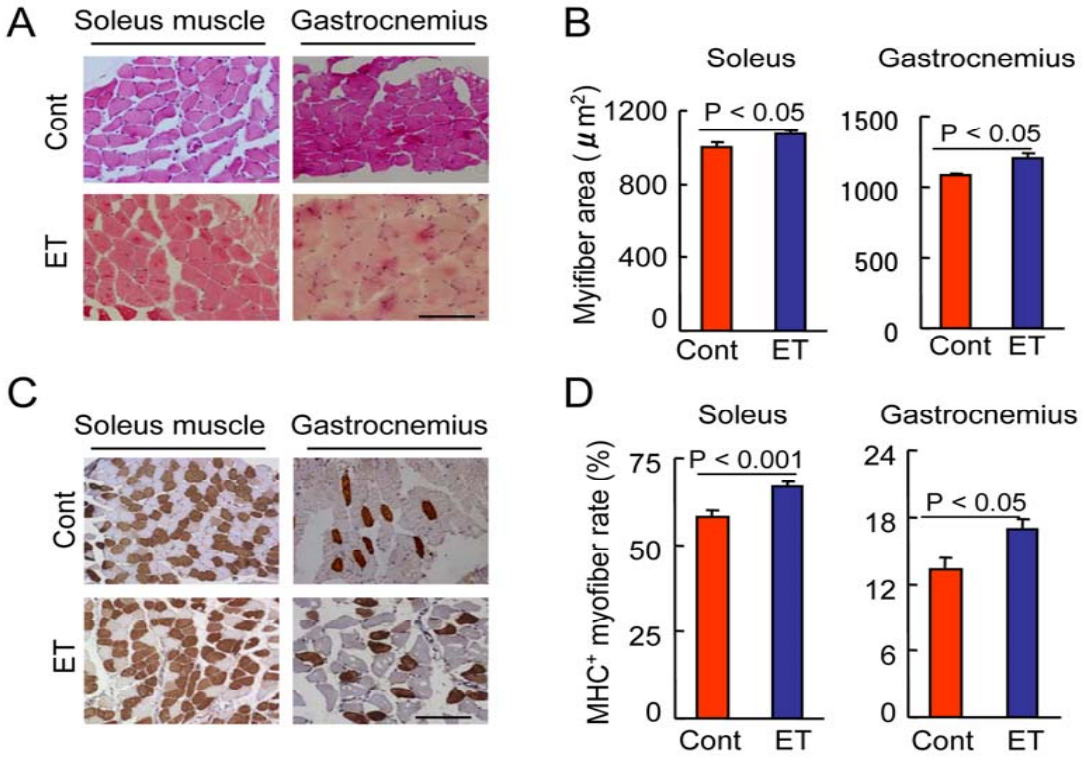
Supplemental Fig. S2



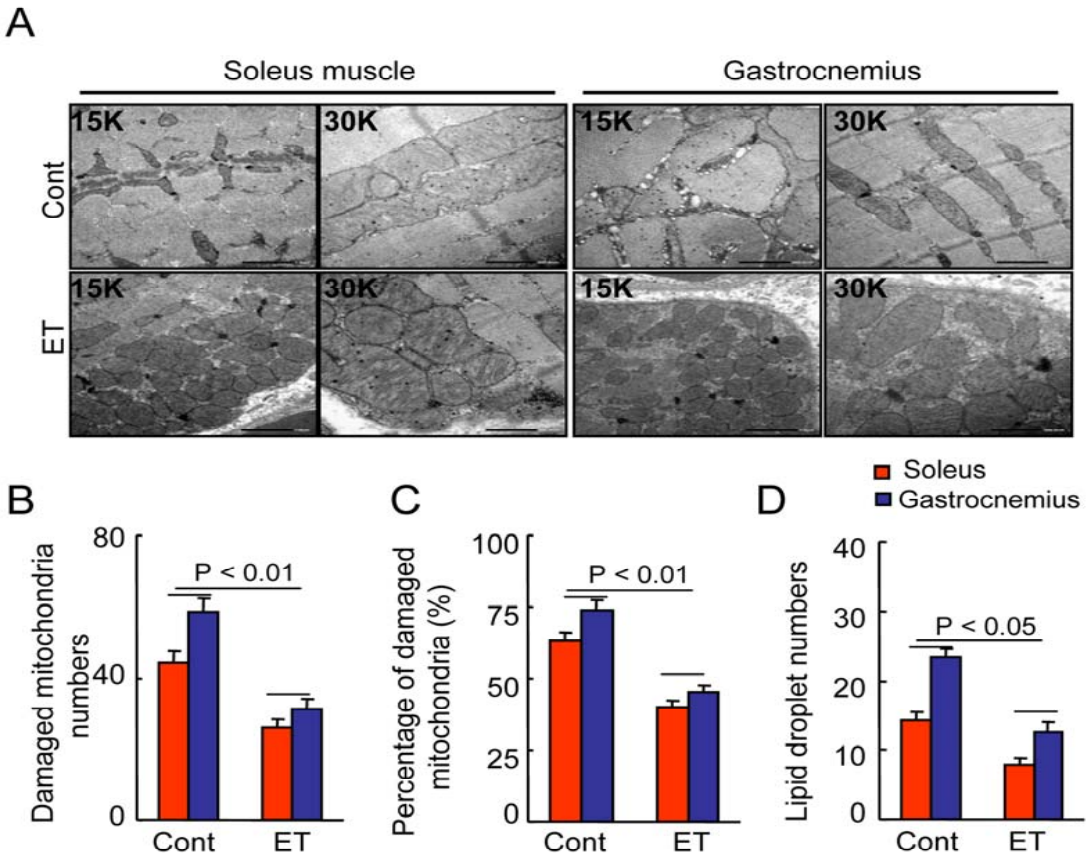
Supplemental Fig. S3



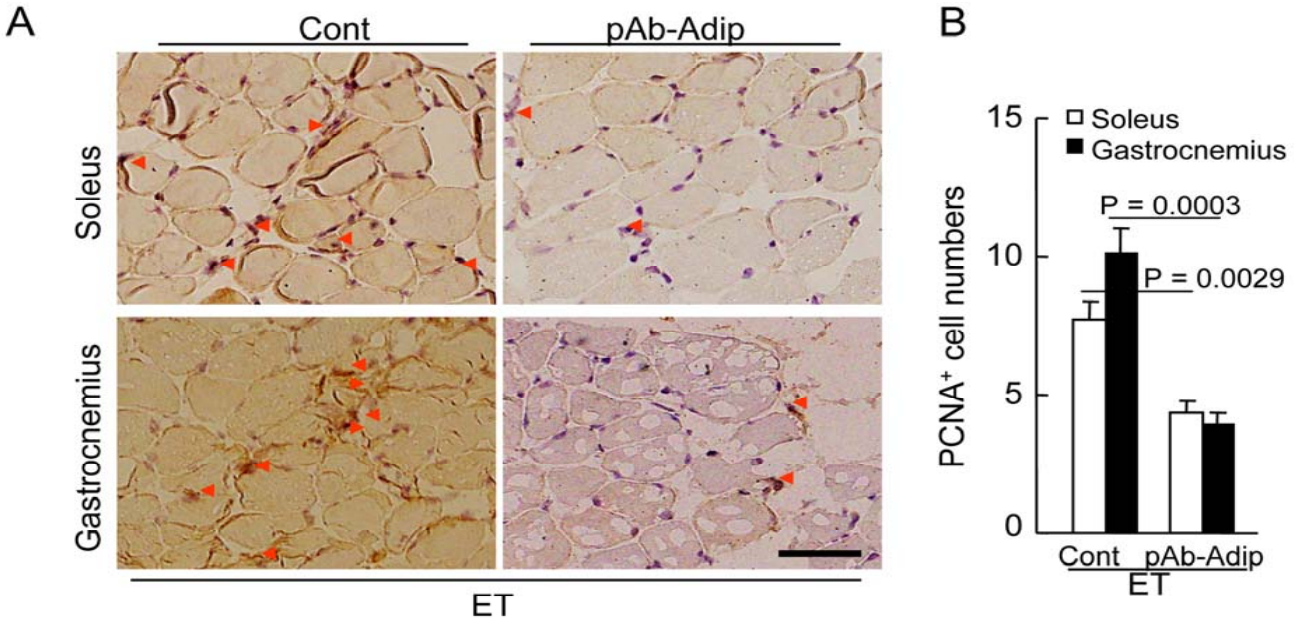
Supplemental Fig. S4



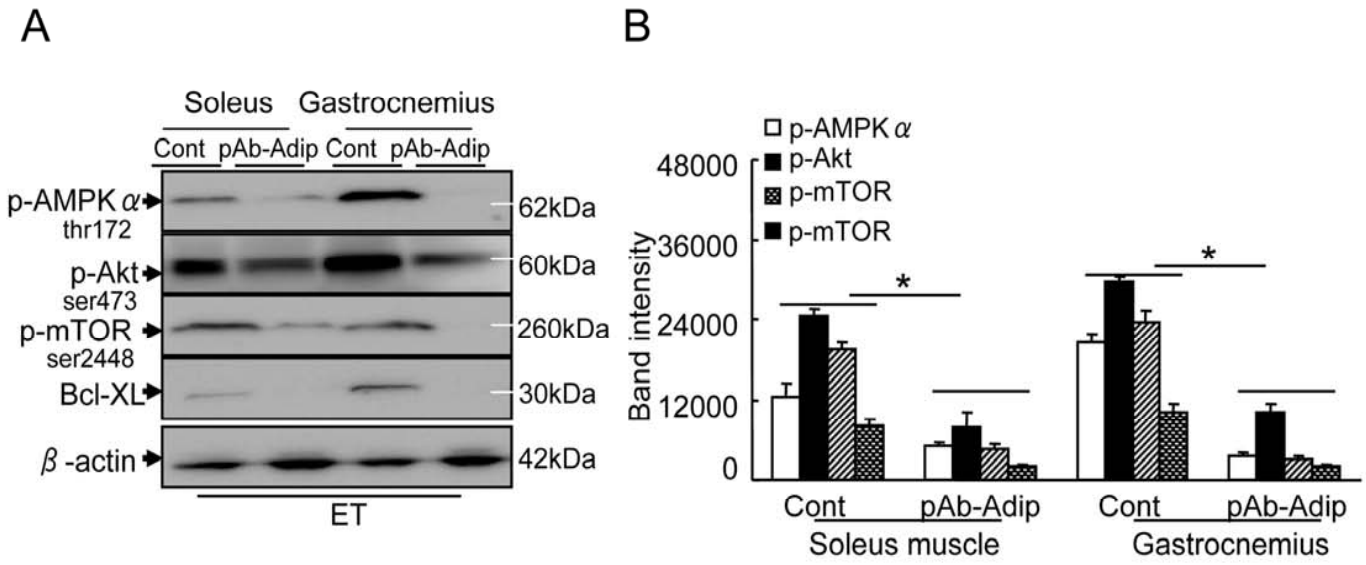
Supplemental Fig. S5



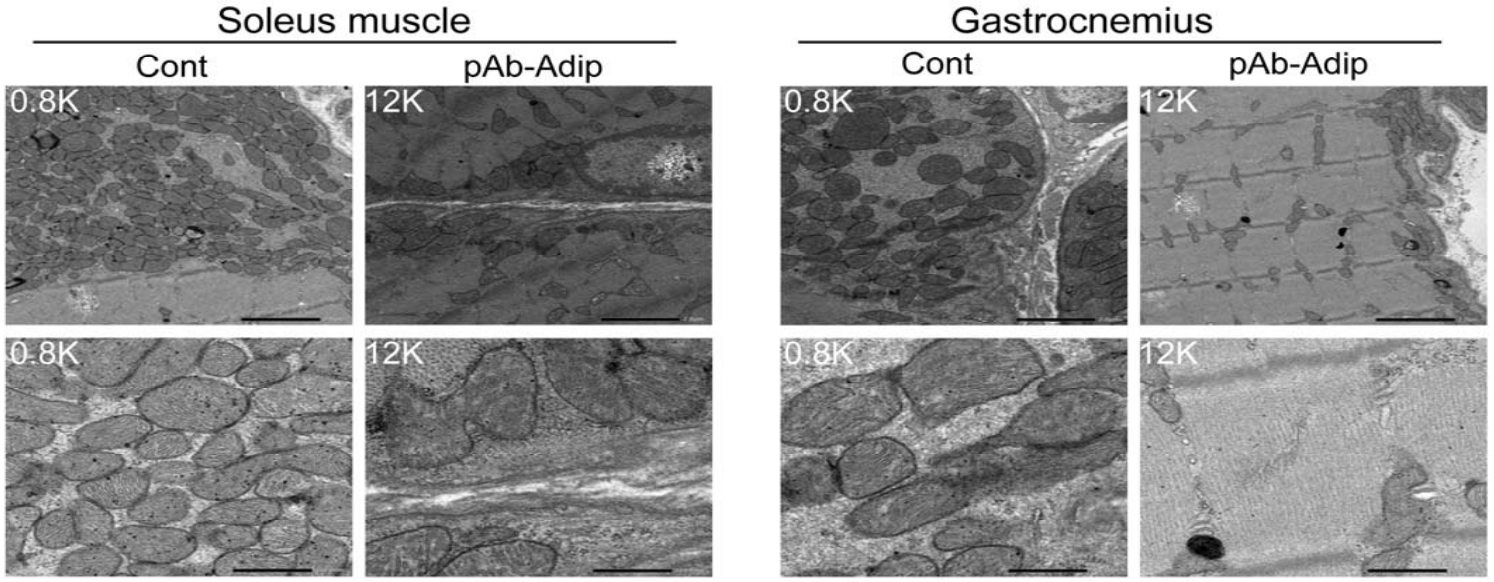
Supplemental Fig. S6



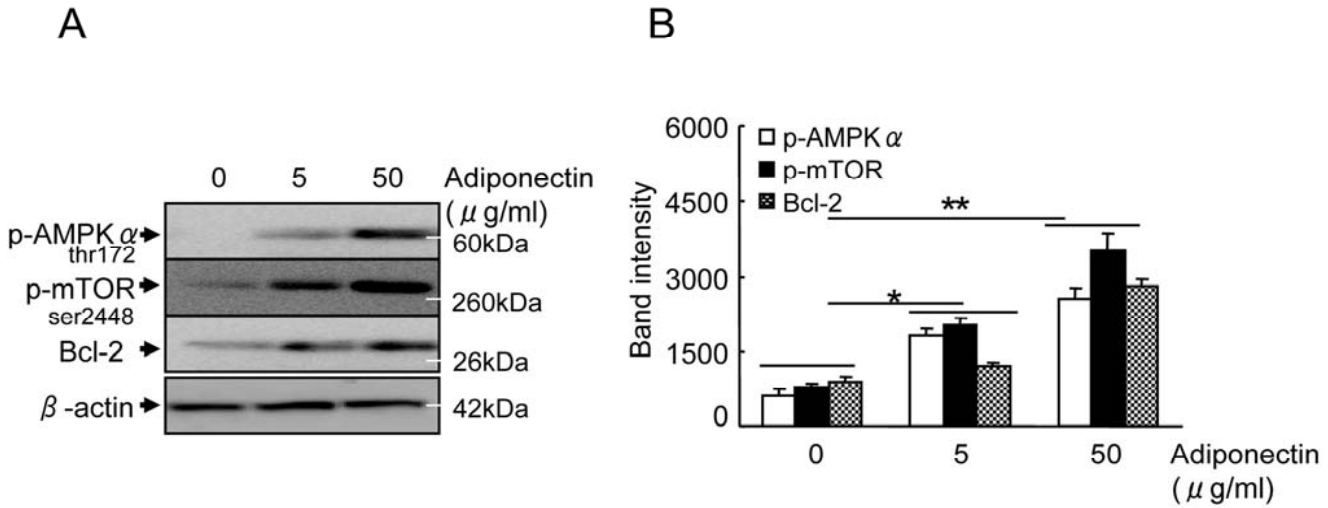
Supplemental Fig. S7



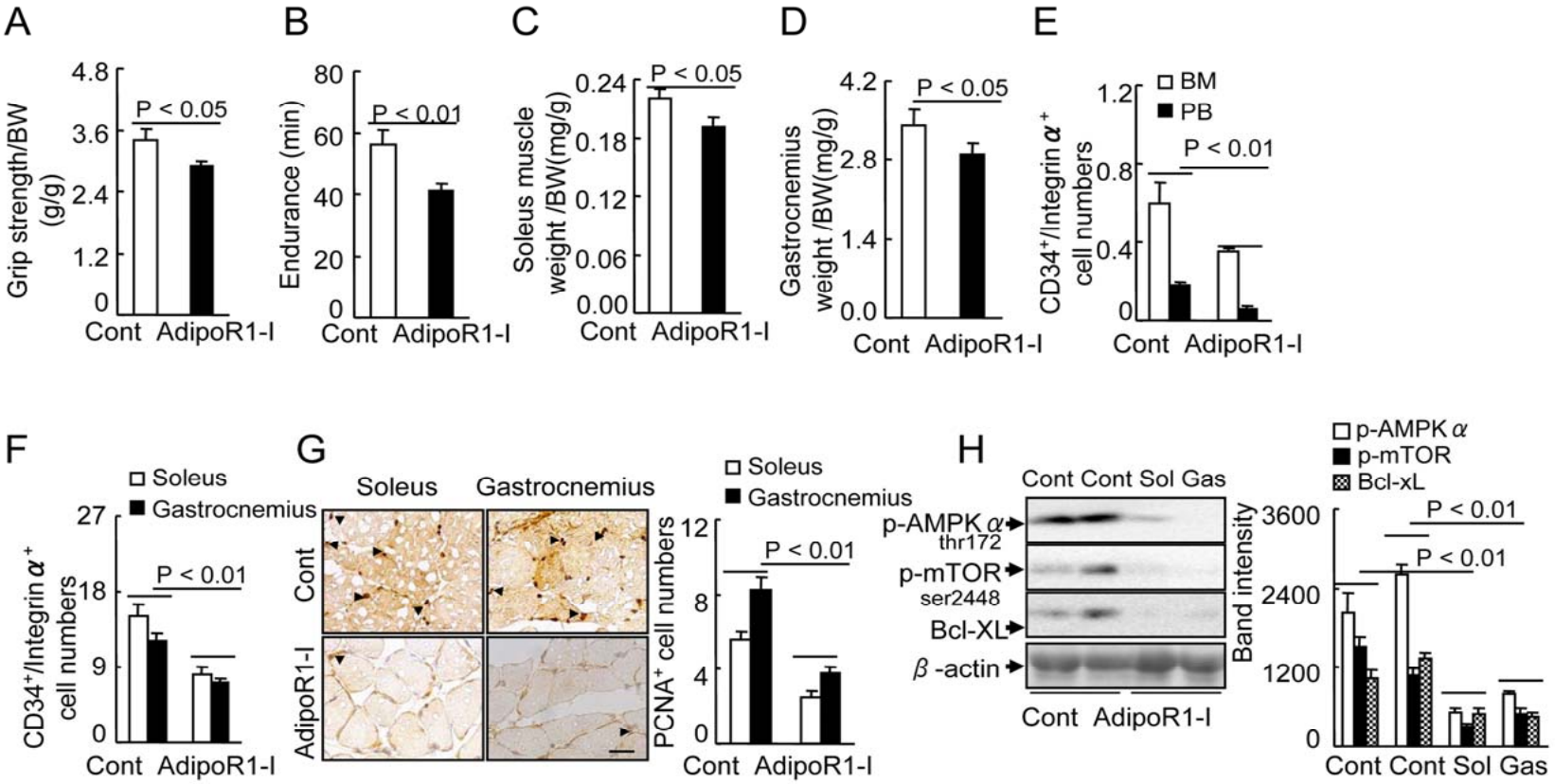
Supplemental Fig. S8



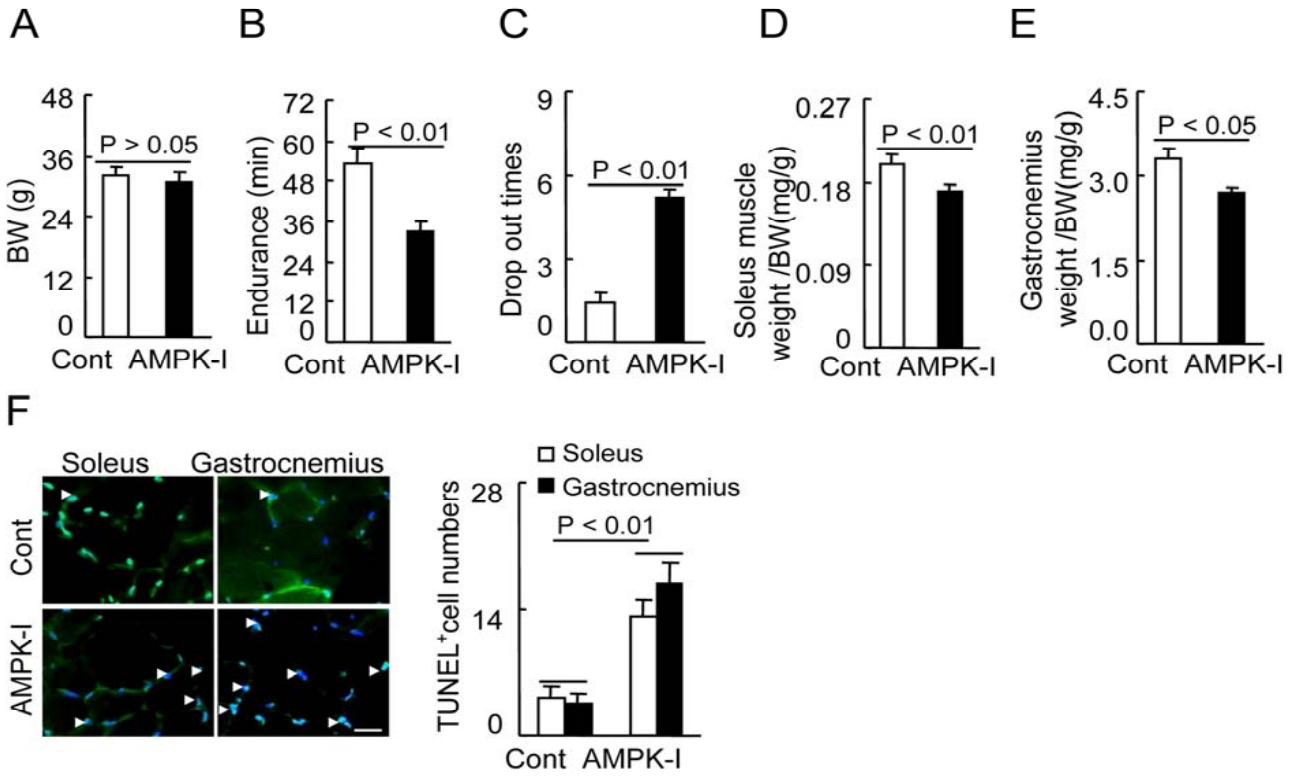
Supplemental Fig. S9



Supplemental Fig. S10



Supplemental Fig. S11



Supplemental Fig. S12