Supporting Information

The hypothalamic-pituitary-gonadal axis controls muscle stem cell senescence through autophagosome clearance

Supporting methods

Luciferase assay

Luciferase assays were performed by seeding 1×10^6 C2C12 cells per well in 6-well plates. Cells were transfected with 5 μg of 4×CLEAR luciferase plasmid constructs and 50 ng of the *LacZ* control vector. Cells were transfected using Metafectine (Biotex), according to the manufacturer's instructions. After 24 h of transfection and inhibitor treatment, the cells were lysed, and luciferase and β-galactosidase activities were measured using a Turner BioSystems luminometer (Promega, Madison, WI, USA).

EdU incorporation assay.

Purified MuSCs were labeled with 5-ethynyl-2´-deoxyuridine (EdU,10 nM, Thermo Fisher Scientific. Mass. USA) for indicated times. EdU-labeled cells were detected by Click iT® Plus EdU assay kit (Thermo Fisher Scientific. Mass. USA) according to the manufacturer's instructions. EdU positive cells were quantified as the percentage of the total number of Hoechst positive cells.

ROS detection assay

To directly monitor ROS production in MuSCs using flow cytometry, live MuSCs were stained with ROS-ID® Total ROS detection kit (Enzo life science. NY. USA) according to the manufacturer's instructions. Flow cytometry analysis software FlowJo was used for MFI measurement. MFI refers to the mean fluorescence intensity of the selected cell population.

Colony formation assay

A total of 5×10^2 freshly isolated MuSCs from indicated mice were plated in 96-well plates. Then, 72-96 h after plating, clusters containing multiple cells (> 5) in each well were counted as individual colonies.

MuSC transplantation

To deplete MuSCs in recipient mice, we orally injected tamoxifen into *Pax7^{CreER};ROSA-DTA* mice for 5 consecutive days. At least 2 weeks after MuSC depletion, the TA muscles of recipient mice were injured with 50 μL of 1.2% $BaCl_2$ 24 h before transplantation. MuSCs were purified from indicated mice by FACS. For lentiviral transduction, we coated 96-well plates with RetroNectin/PBS (final concentration of 20 μg/mL). Freshly isolated WT, dKO, or geriatric MuSCs and pLVX-Tfeb-IRES-Tdtomato or pLVX-mock-IRES-Tdtomato lentivirus were plated in the RetroNectin coated 96-well plates and incubated for 16 h at 37°C in a cell culture

incubator. We resuspended 1×10^4 MuSCs in PBS and transplanted them into the TA muscles of MuSC-depleted *Pax7^{CreER};ROSA-DTA* mice that had been injured with $BaCl_2$ 24 h before. Engrafted muscles were collected 10 days after transplantation, embedded in OCT compound, and stored frozen at –80°C until analysis. To minimize experimental variation, we transplanted control MuSCs into the pre-injured contralateral TA muscles in the same mice.

Immunohistochemistry

Immunohistochemistry was performed on muscle cryosections using the following steps: 7-μm sections were fixed in 4% paraformaldehyde for 10 min, washed in PBS, and treated in MOM blocking solution, according to the manufacturer's instructions (FMK-2201; Vector Laboratories). The sections were blocked for 1 h at room temperature with 4% bovine serum albumin in PBS and incubated with primary antibodies overnight at 4 ℃. The slides were washed with PBS several times and incubated with the appropriate secondary antibodies for 1 h at room temperature. The slides were counterstained with Hoechst and mounted with Vectashield (H-1000; Vector Laboratories) after washing with PBS. CSA was quantified on muscle cross-sections following laminin staining. At least 50 field images were acquired from fifteen cross-section slides of each muscle and measured using image analysis software (Leopard). Each dot shows that the averaged data of mean CSA from one muscle (n=4-7). Each muscle was isolated from at least 4 mice per group. The averages of mean CSA in each muscle were statistically analyzed. For quantifying relative

percentages of myogenic marker positive cells at 3 dpi, the regeneration area was determined by centrally nucleated myofibers in captured fields. Each dot is relative percentages of positive cells in at least 30 regeneration area from one mouse (n=4). The relative percentages of marker positive cells per regeneration area were statistically analyzed.

RNA extraction and measurement of mRNA expression

Total RNA was extracted from freshly isolated MuSCs using an RNeasy Micro Kit (Qiagen, GmbH, Hilden, Germany) or TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) and analyzed by qRT-PCR. First-strand complementary DNA was synthesized from 1 μg of RNA using ReverTra Ace (Toyobo, Osaka, Japan) according to the manufacturer's instructions. qRT-PCR (Qiagen) was performed with SYBR Green technology (SYBR Premix Ex Taq, Qiagen) using specific primers against indicated genes.

The following mouse primers were used in this study. *p16*^{Ink4a}; forward (fwd) 5'-CTTCTGCTCAACTACGGTGC-3', reverse (rev) 5'- GCACGATGTCTTGATGTCCC-3'; *p21*^{Cip1}; fwd 5'-ACAAGAGGCCCAGTACTTCC-3', rev 5'-GGGCACTTCAGGGTTTTCTC-3'; *Lamin B1*; fwd 5'- CCTGGAAGGCGAAGAAGAG-3', rev 5'- CTGAGGCAGACTGGGAAATG-3';

Gapdh; fwd 5'- ACACATTGGGGGTAGGAACA-3', rev 5'-AACTTTGGCATTGTGGAAGG-3'; *Tfeb*; fwd 5'-CCACCCCAGCCA TCAACAC-3', rev 5'-CAGACAGATACTCCCGAACCTT-3'; 18s rRNA; fwd 5'-

CCTCCAATGGATCCTCGTTA-3', rev 5'- AAACGGCTACCACATCCAAG-3'; Beclin1; fwd 5'-ATGGAGGGGTCTAAGGCGTC-3', rev 5'-TCCTCTCCTGAGTTAGCCTCT-3'; M6pr; fwd 5'-TGCTGGAGGACTGAACTGTTA-3', rev 5'-GAGCCACCTCGTTCTTTGACT-3'; Vps18; fwd 5'-TTGCTCAAGATCGAGGATGTGC-3', rev 5'-CCTGCAAGTCTCGTCGGATG-3'; Ulk1; fwd 5'-AAGTTCGAGTTCTCTCGCAAG-3', rev 5'-ACCTCCAGGTCGTGCTTCT-3'; Neu1; fwd 5'-GGACCGCTGAGCTATTGGG-3', rev 5'-CGGGATGCGGAAAGTGTCTA-3'; Bnip3; fwd 5'-GCACTTCAGCAATGGCAATGG-3', rev 5'-GCTACTTCGTCCAGATTCATGC-3'; Maplc3; fwd 5'-TTATAGAGCGATACAAGGGGGAG-3' rev 5'-CGCCGTCTGATTATCTTGATGAG-3'; sqstm1; fwd 5'-AGGATGGGGACTTGGTTGC-3', rev 5'-TCACAGATCACATTGGGGTGC-3'; Ctns; fwd 5'-ATGAGGAGGAATTGGCTGCTT-3', rev 5'-ACGTTGGTTGAACTGCCATTTT-3'; Mcoln1; fwd 5'-CTGACCCCCAATCCTGGGTAT-3', rev 5'-GGCCCGGAACTTGTCACAT-3'; Lamp1; fwd 5'-CAGCACTCTTTGAGGTGAAAAAC-3', rev 5'-ACGATCTGAGAACCATTCGCA-3'; *Ar*; fwd 5'-CGAAGTGTG GTATCCTGGTG-3', rev 5'-AAAACATGGTCCCTGGTACTG-3'; *Esr2* (isoform 1); fwd 5'-CCAAGCAGGAAGAAAGAGGA-3', rev 5'-TGGACTGTAGAACGGTGTGG-3'; *Esr2* (isoform 2) fwd 5'- CCAAGCAGGAAGAAAGAGGA-3', rev 5'-TGGACTGTAGAACGGTGTGG-3'; 18s rRNA; fwd 5'- CCTCCAATGGATCCTCGTTA-3', rev 5'- AAACGGCTACCACATCCAAG-3'; *Hes1*; fwd 5'-AGGCGCAATCCAATATGAAC-3', rev 5'- CCCACCTCTCTCTTCTGACG-3'; *Hey1*; fwd 5'-GCTCGTATGTCTGGTGCTGA-3', rev 5'- CACTCCCTGAAGACGAAAGC-3';

HeyL; fwd 5'-GCTCGTATGTCTGGTGCTGA-3', rev 5'-CACTCCCTGAAGACGAAAGC-3'. The thermocycling conditions were as follows: 10 min at 95°C, followed by 40 cycles of denaturation for 5 s at 95°C and annealing/extension for 30 s at 60°C. The reactions were run in triplicate, and automatically detected threshold cycle (Ct) values were compared between samples. Relative quantification of gene expression was performed using the ΔΔCt method. Transcripts of the β-actin, *Gapdh*, or 18S rRNA housekeeping genes were used as endogenous controls.

Immunoblotting analysis

Muscle stem cells were homogenized in RIPA buffer (50 mM Tris-HCl, pH 7.5, 0.5% SDS, 20 μg/mL aprotinin, 20 μg/mL leupeptin, 10 μg/mL phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, and 1 mM dithiothreitol). Bradford's reagent (Bio-Rad Laboratories, Hercules, CA, USA) was used for estimating total protein concentrations. Total proteins were analyzed by electrophoresis in 8–15% polyacrylamide gels and transferred to PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked in 5% skim milk (BD Biosciences) in 0.1% Tween-20 TBS and incubated with primary antibodies overnight at 4°C. After incubation with the corresponding HRP-conjugated secondary antibodies (Promega), the membranes were developed using the Fusion solo chemiluminescence imaging system (Vilber, Marne-la-Vallée, France). GAPDH, β-tubulin, or β-actin was used as a loading control.

ChIP

For ChIP analysis of AR, 3×10^6 FACS-purified MuSCs from healthy young mice were crosslinked in 1% formaldehyde (Sigma-Aldrich) for 10 min. Crosslinking was quenched with 0.1 M glycine for 5 min, and cells were washed three times with ice-cold PBS. Cells were lysed in lysis buffer containing 50 mM Tris-HCl (pH 8.1), 10 mM EDTA, and 1% SDS supplemented with a complete protease inhibitor cocktail (Roche, Basel, Switzerland), and the chromatin was sonicated. Sheared chromatin was cleared by centrifugation at 17,000 ×*g* for 10 min, and 2% of the chromatin was used as the input fraction. Chromatin was immunoprecipitated overnight with 20 µL protein A/G beads (GE Healthcare) pre-coupled with 1 µg AR antibody (Abcam, ab74272) in ChIP dilution buffer. Beads were washed three times with low-salt wash buffer [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), 150 mM NaCl], high-salt wash buffer [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), 500 mM NaCl], buffer III [0.25 M LiCl, 1% NP-40, 1% deoxycholate, 10 mM Tris-HCl (pH 8.1), 1 mM EDTA], and TE buffer [10 mM Tris-HCl (pH 8.0), 0.5 M EDTA] and eluted in elution buffer (1% SDS, 0.1 M $NaHCO_3$). The supernatant was incubated overnight at 65°C for reverse-crosslinking and then digested with RNase A for 2 h at 37°C and proteinase K for 2 h at 55°C. Reverse-crosslinking and elution were performed in a volume of 1 mL. DNA was then purified and analyzed with qRT-PCR analysis using specific primers for the androgen response element (ARE) in the *Tfeb* promoter region. The following primers were used in ChIP assays. ARE in Ar promoter; fwd 5'-ATCAGAAGGATCATTGGGGT-3', rev 5'-TGT CCTTAGAGA AAGGTTGT-3'; ARE #1; fwd 5'-GGATTTGAGATGGCCTTGAA-3', rev 5'-GGTTGCCAA

TAAGGAAGCTG-3'; ARE #2; fwd 5'- ATGGTGGCTCACAACCATCT-3', rev 5'-CATCTC CTTGAGCCTTCTGG-3'; ARE #3; fwd 5'-CCTTCTGCTAGCCCACAAAC-3', rev 5'- GTGAAAACTGGGGACAAGGA -3'; ARE #4; fwd 5'-TGGGCATGGAGAGCTTATTT-3', rev 5'-AGCTGGAGAGGAGTCAGCAG -3'; Untr6 (Neg.); fwd 5' TCAGGCATGAACCACCATAC-3', rev AACATCCACACGTCCAGTGA.

RNA-seq

The TruSeq method was used to generate Illumina RNA-seq libraries, according to the manufacturer's instructions. RNA-seq libraries were sequenced as paired-ends on an Illumina Hi-Seq 4000. Differentially expressed genes were identified using a false discovery rate (FDR) cut-off of 1×10^{-5}. We performed hierarchical clustering analysis using the gene expression values from wild-type vs. *Pax7-CreER;Ar$^{f/y}$;Esr2$^{-/-}$* mice using the Euclidean method and complete linkage. RNA sequencing data sets have been deposited in the NCBI Gene Expression Omnibus (GEO) database under the accession number GSE121016.

Ingenuity Pathway Analysis

The Ingenuity Pathway Analysis (IPA, Qiagen) tool was used for bioinformatic prediction of upstream regulator candidates, obtained using autophagy- and lysosome-related genes showing a –1.5-fold change in expression in dKO MuSCs compared to WT MuSCs. Technical requirements are available from the IPA website

(www.ingenuity.com).

Measurement of serum steroids

To measure circulating sex steroid levels, serum samples were taken from each group and analyzed by GC-MS/MS as previously described [1]. In brief, to prepare the serum, whole blood was collected through retro-orbital bleeding and placed into 1.5-mL Eppendorf tubes. The blood was clotted by incubating at room temperature for 30 min, and the serum was collected after centrifugation for 30 min at 3,000 rpm and stored at −80°C until use. The serum was spiked with 15 μL of IS mixture and diluted with 200 μL of acetate buffer (pH 5.2). The sample was completely soaked into the sorbent and then allowed to stand for 5 min for adsorption on diatomaceous earth-supported material. Each glass tube was placed under an SLE plate in a vacuum manifold, and the sample was extracted three times with 0.8 mL of dichloromethane (DCM). After elution from the sorbent by gravity, the SLE cartridge was applied to vacuum at below 0.2 bar for 30 s to finish the extraction. The organic solvent was evaporated in an N_2 evaporator at 40°C and dried in a vacuum desiccator over P_2O_5-KOH for at least 30 min. Finally, the dried residue was derivatized with 30 μL of a mixture of MSTFA/NH_4I/DTE (500:4:2, v/w/w) at 60°C for 20 min and then 2 μL was injected into the GC-MS system. The extraction efficiencies of elution solvents, such as *t*-butyl methyl ether (MTBE), diethyl ether, ethyl acetate (EA), a mixture of DCM and EA (4:1, v/v), and a mixture of DCM and isopropyl alcohol (IPA; 95:5, v/v), were compared. GC-MS analysis was performed with a Thermo trace gas chromatograph interfaced with a

triple-quadrupole TSQ Quantum XLS mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA). To perform the quantitative analysis, the characteristic ions and retention times of 37 steroids, as TMS derivatives, were determined in the full scan (*m/z* 100 to 750), and then both SIM and SRM modes were applied to analyze all steroids and ISs in 12 different segments.

Senescence-associate β-galactosidase assay

Senescence-associate β-galactosidase (SA-β-Gal) activity was detected in MuSCs using the senescence β-galactosidase staining kit (Enzo, Farmingdale, NY, USA), according to the manufacturer's instructions.

Antibodies

The following antibodies were used in this study: Anti-Pax7 (DSHB, PAX7), Anti-Laminin (Abcam, ab11576), Anti-p62 (Abcam, ab101266), Anti-γH2AX (Abcam, ab111174), Anti-Androgen receptor (Abcam, ab74272), Anti-eMyHC (DSHB, BF-G6), Anti-Gapdh (Cell signaling, 14C10), Anti-poly-ubiquitin (Enzo, FK1), Anti-CD45-APC (Biolegend, 30-F11), Anti-CD31-APC (Biolegend, MEC13.3), Anti-Sca1-PE (Biolegend, E13-161.7), Anti-Vcam1-biotin (Biolegend, 429 MVCAM)

Optical systems

Immunofluorescence was performed using a Zeiss Observer Z1 fluorescent microscope (Zeiss, Oberkochen, Germany) equipped with a SPOT Flex camera or a Zeiss confocal system LSM710. For myofiber diameter measurements, Leopard morphometric software (INS Industry, Seoul, Korea), Spot software (version 5.1, Diagnostic Instruments, Sterling Heights, MI, USA) and Photoshop CS6 (Adobe) were used.

Supporting data (Figure S1-S7)

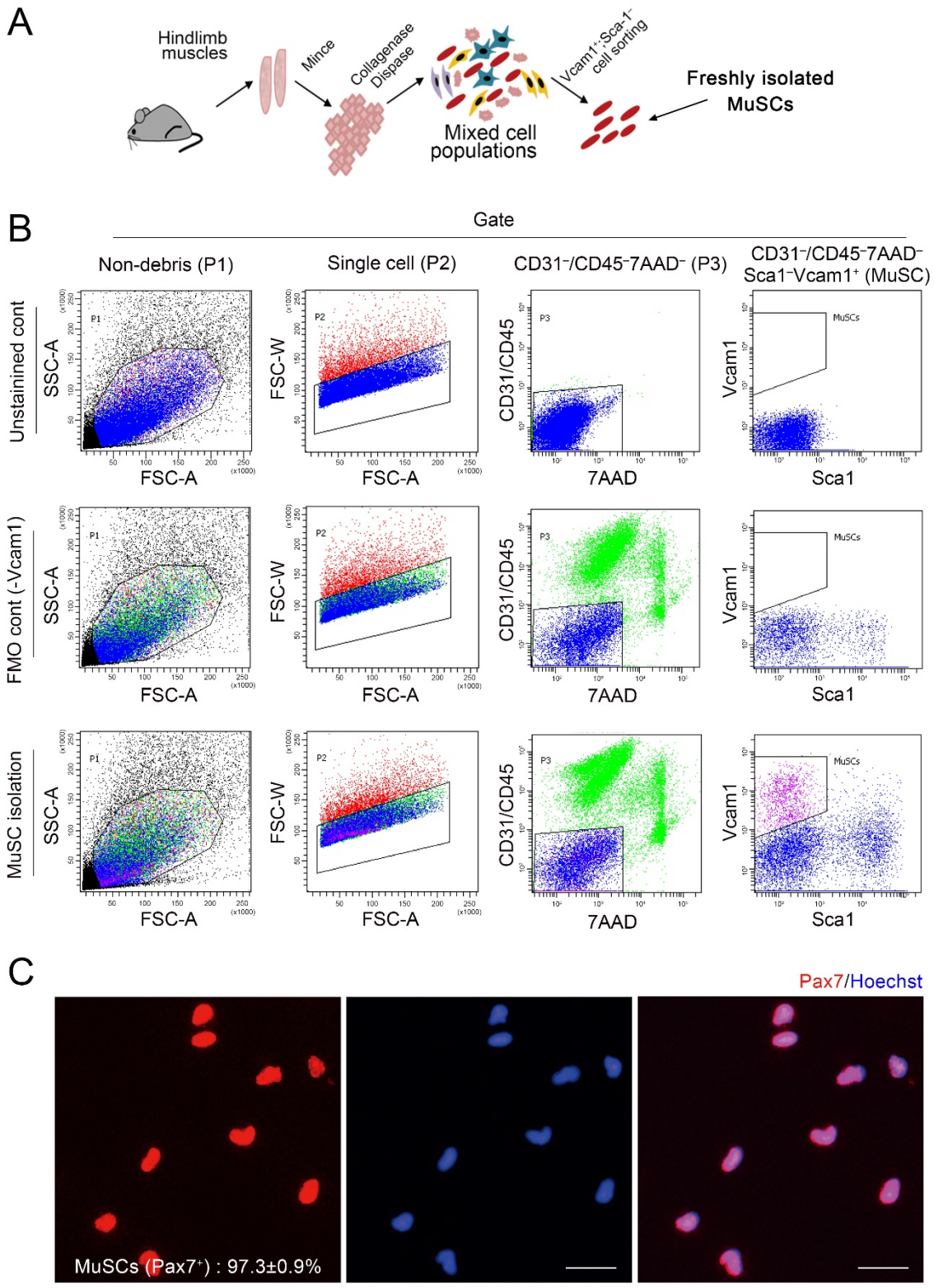

Figure S1. Flow cytometry isolation protocol for MuSCs.

(**A**) A scheme for isolation of MuSCs. (**B**) The gating strategy and representative FACS profiles of adult quiescent MuSCs. (**C**) Freshly isolated $Lin^{-}Vcam^{+}Sca1^{-}$ cells were stained with an anti-Pax7 antibody. More than 97% of freshly isolated $Vcam^{+}$ cells were $Pax7^{+}$ indicating that most of them were MuSCs. Scales, 25 μm (C).

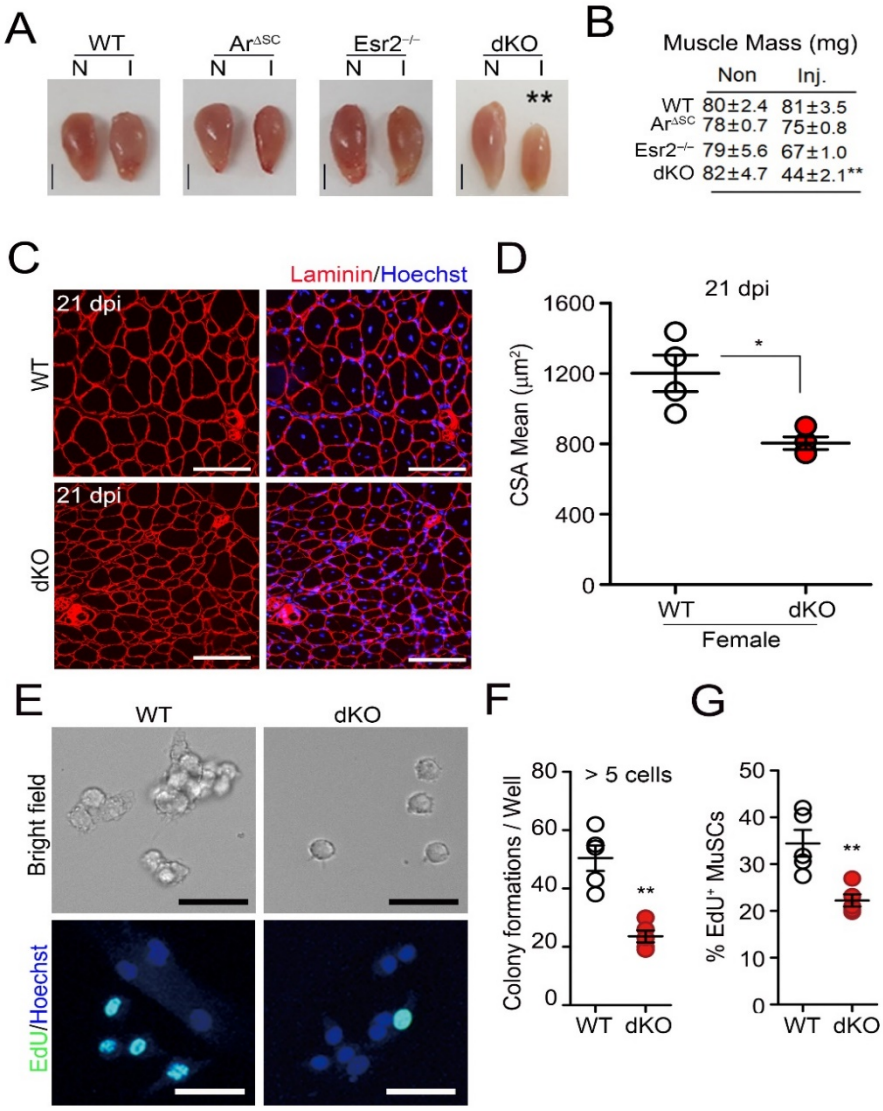

Figure S2. Impaired skeletal muscle regeneration and MuSC function in the absence of MuSC intrinsic sex steroid receptor signaling

(**A-B**) Three-month-old male wild-type (WT), Pax7^{CreER};Ar$^{f/y}$ (Ar$^{\Delta SC}$), Esr2$^{-/-}$ and Pax7^{CreER};Ar$^{f/y}$;Esr2$^{-/-}$ (dKO) mice were orally administered with tamoxifen (Tmx) for 5 consecutive days. Tibialis anterior (TA) muscles were injured by BaCl2 injection at 6

months after Tmx administration. TA muscles were analyzed at 21 days post- $BaCl_2$ injury (dpi). Gross morphology (A) and muscle mass of non-injured; N and injured; I TA muscles (B). (**C-D**) TA muscles of female WT and dKO mice were injured 6 months after Tmx administration. Immunohistochemical (IHC) images of laminin staining at 21 dpi (C) and quantification of mean cross-section-area (CSA) (D) for regenerated TA muscle. The detailed procedure of CSA quantification applied throughout this study is described in the Supporting information. (**E**) Representative images of colony-forming (top) and 5-ethynyl-2´-deoxyuridine (EdU) incorporation (bottom) assays of purified MuSCs 96 hours after plating. (**F**) The quantification of the number of colony formation and (**G**) EdU+ MuSCs. Cell clusters consisting of at least 5 or more cells were counted as a colony. Comparisons by Kruskal–Wallis one-way analysis of variance (D) and unpaired *t*-test (F and G). Scales, 50 (E), 100 μm (C), and 0.5 cm (A). Bars, mean ± s.e.m.; n = 4-5 animals per group; n.s, non-significance.

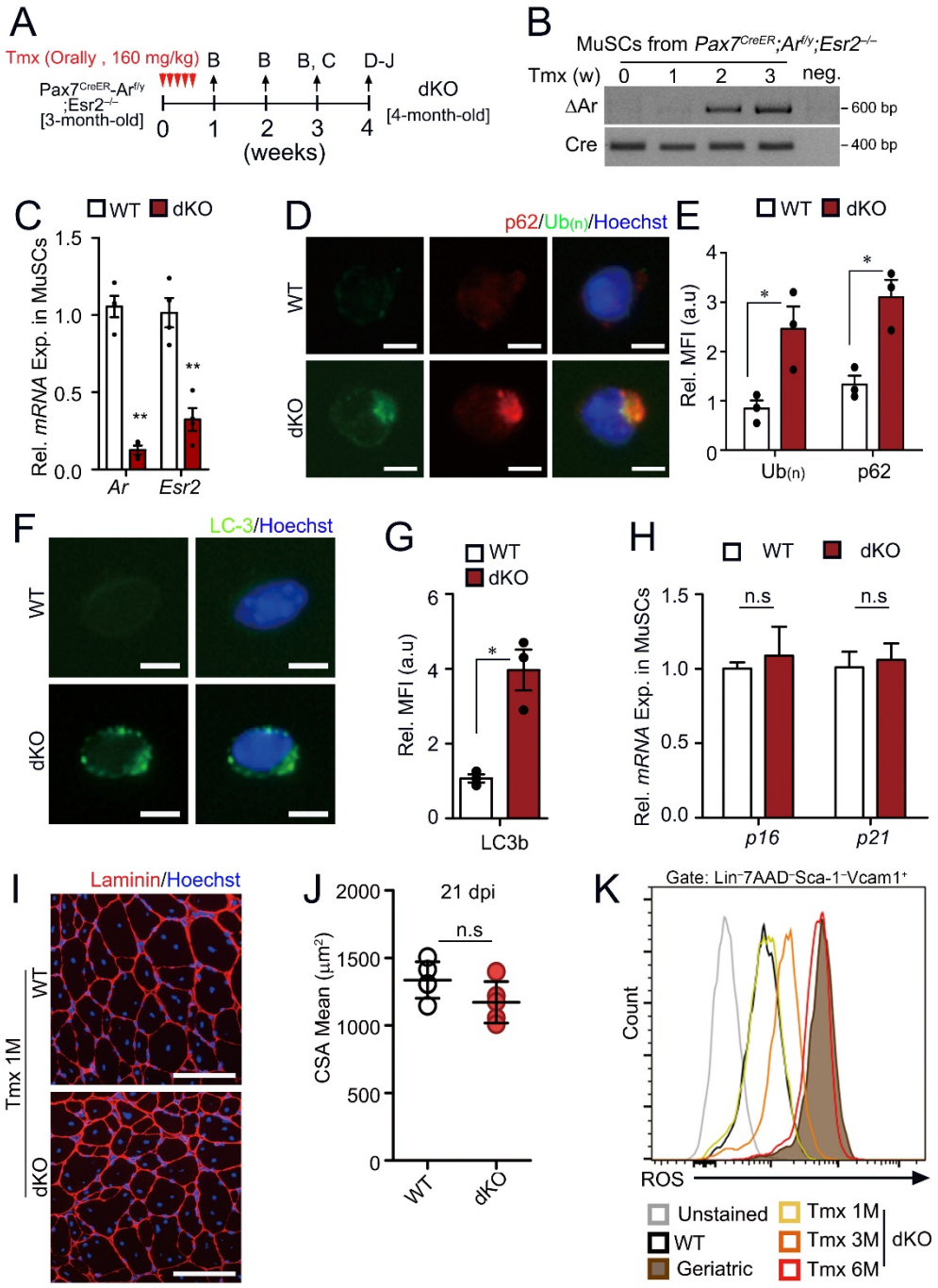

Figure S3. Defective autophagy flux and increased ROS in sex hormone receptor signaling disrupted MuSCs.

(**A**) A scheme for Tmx administration. (**B**) PCR analysis to evaluate the efficacy of Tmx on MuSC-specific genomic deletion of the androgen receptor. $CD31^{-}CD45^{-}Sca1^{-}$

$7AAD^{-}Vcam1^{+}$ MuSCs were isolated from dKO mice at indicated weeks after Tmx administration. $CD31^{-}CD45^{-}Sca1^{-}7AAD^{-}Vcam1^{-}$ cells from the same mice were used as negative controls. (**C**) Relative mRNA expressions of Ar and Esr2 in MuSCs from WT and dKO mice 3 weeks after Tmx treatment. (**D-H)** MuSCs were isolated from WT and dKO mice at 4 weeks after Tmx administration and were analyzed. Representative immunostaining images for p62 and poly-ubiquitin proteins (D) and quantification of mean fluorescence intensity (MFI) (E). Representative immunostaining images (F) for LC3 proteins and quantification (G). Relative expressions of senescence markers, *p16* and *p21*, in WT and dKO MuSCs (H). (**I** and **J**) TA muscles were injured by $BaCl_2$ injection 1 month after Tmx administration. TA muscles were analyzed at 21 dpi. Immunohistochemical (IHC) staining for laminin (I) and mean CSA of regenerated myofibers (J). (**K**) Flow cytometric histogram for reactive oxygen species (ROS) in MuSCs. dKO MuSCs were analyzed at 1, 3, or 6 months after Tmx administration. WT and geriatric MuSCs were isolated from 9-month and 30-month old mice, respectively. Comparisons by unpaired *t*-test (J) and Mann-Whitney u test (C, E, G, H). Scales, 10 μm (D and F), 50 μm (I). Bars, mean ± s.e.m.; n = 4 animals per group; *P<0.05, **P<0.01, n.s, non-significance.

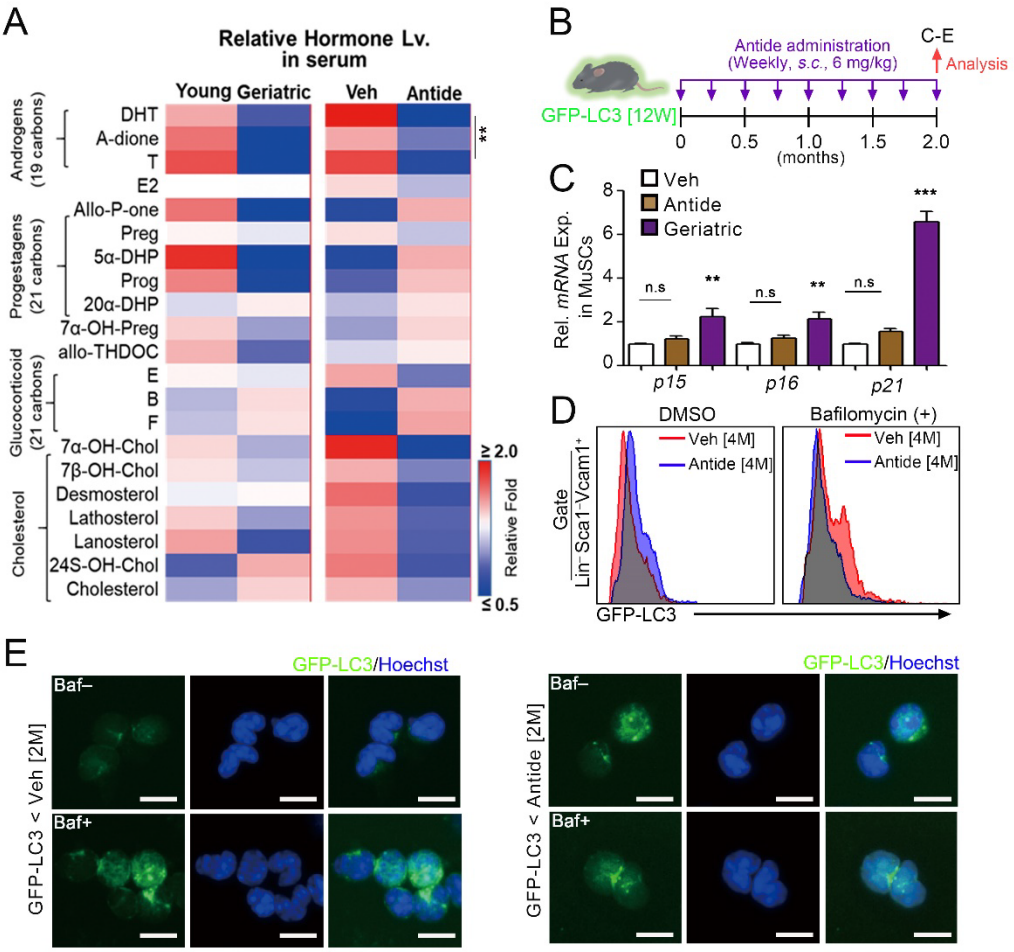

Figure S4. Alteration of autophagy flux by disruption of HPG axis

(**A**) Relative serum steroid profiling by GC-MS/MS. The young and old mice were 4 months and 30 months, respectively. The HPG axis was disturbed by the administration of Veh or Antide to 3-month-old mice for 10 months. Representative mass-spectrometric chromatogram and the result of steroid profiling for blood samples were present in Table S1. (**B**-**E**) Three-month-old GFP-LC3 transgenic mice were administered Veh or Antide for 2 months. A scheme for Antide administration to GFP-LC3 transgenic mice (B). Relative mRNA expressions of p15, p16, and p21 in the MuSCs. Geriatric mice were 30 months of age (C). Flow cytometric histograms (D)

and representative images (E) of GFP-LC3 in MuSCs treated with or without Bafilomycin (Baf+ or Baf–) for 4 hours. Scales, 10 μm (E). Comparisons by one-way ANOVA with Tukey's post hoc test. Bars, mean ± s.e.m.; n = 4 (B), 3 (C and D) animals per group; n.s, non-significance, *P<0.05, **P<0.01.

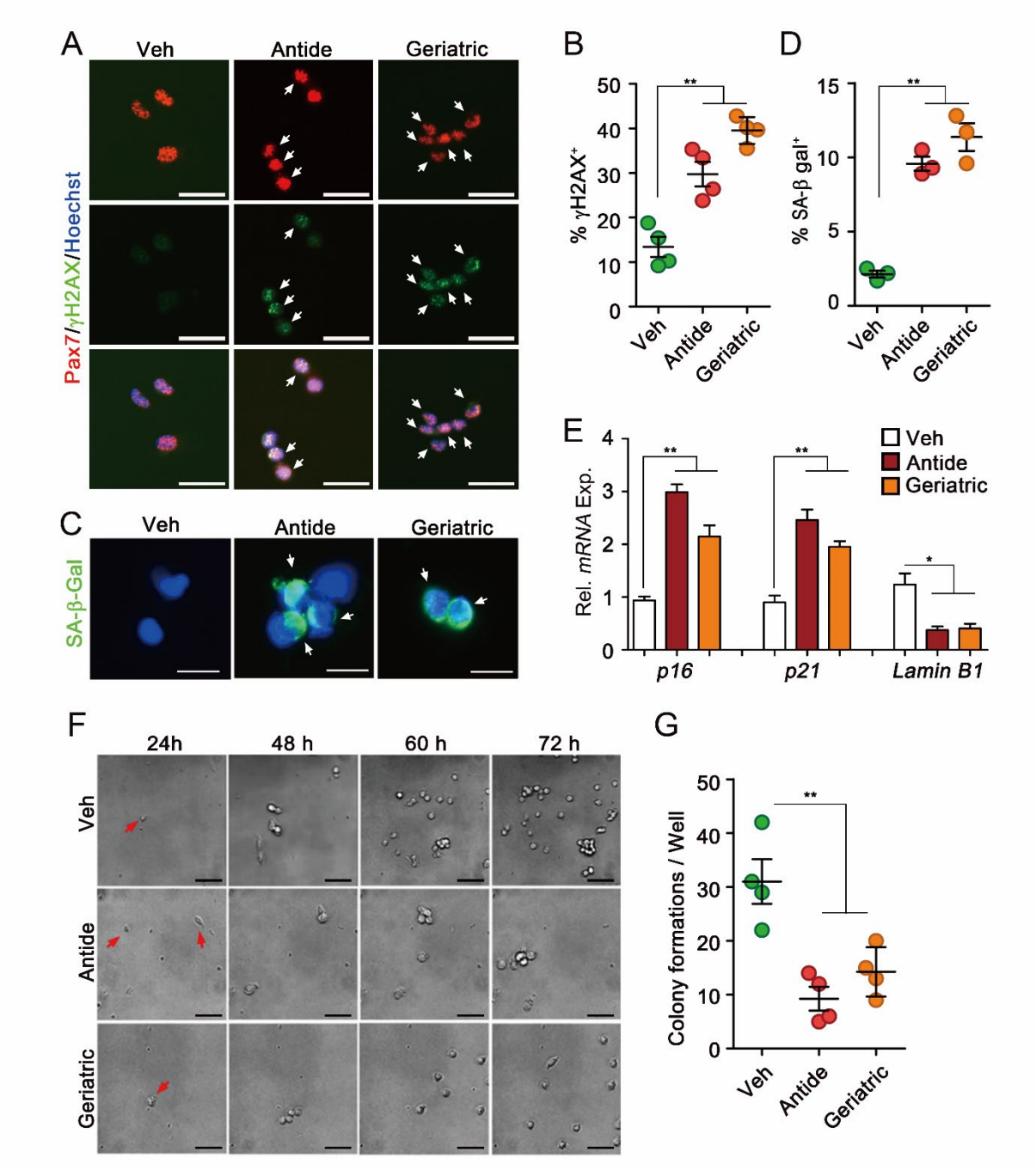

Figure S5. Enhanced DNA damage and senescence of MuSCs by Antide administration

(**A**-**E**) Three-month-old mice were subcutaneously injected with Veh or Antide for 6 (A

and B) or 10 (C-E) months. Geriatric mice were 30 months old. Immunostaining (A) against Pax7 and γH2AX and quantification (B) of $Pax7^{+}\gamma H2AX^{+}$ MuSCs (Arrows) freshly isolated from Veh- or Antide-treated mice. Representative images (C) and quantification (D) of SA-β-Gal^{+} cells (Arrows) in MuSCs isolated from Veh- or Antide-treated mice. Relative mRNA expression of senescence-associated genes in MuSCs (E). (**F**) Representative live-cell images and (**G**) quantification of colony formation of *ex vivo* cultured MuSCs freshly isolated from 13-month-old Veh-, Antide-treated, or geriatric mice. Scales, 50 μm (A and F), and 20 μm (C). Comparisons by one-way ANOVA with Tukey's post hoc test. Bars, mean ± s.e.m.; n = 4 animals per group; *P<0.05, **P<0.01.

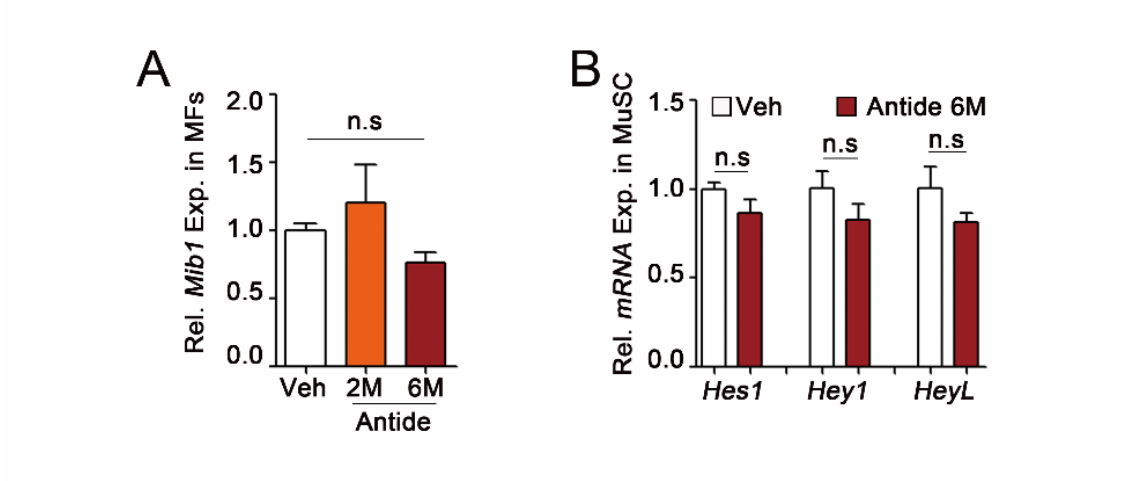

Figure S6. Reduced levels of sex steroid hormones by Antide treatment might be insufficient to block Mib1-Notch signaling

Three-month-old mice were subcutaneously injected with Veh or Antide for indicated periods. (**A**) Relative mRNA expression of *Mib1* in myofibers. (**B**) Relative mRNA expression of Notch target genes in MuSCs. Comparisons by one-way ANOVA with Tukey's post hoc test or two-tailed *t*-test. Bars, mean ± s.e.m.; n = 3, n.s. not significant.

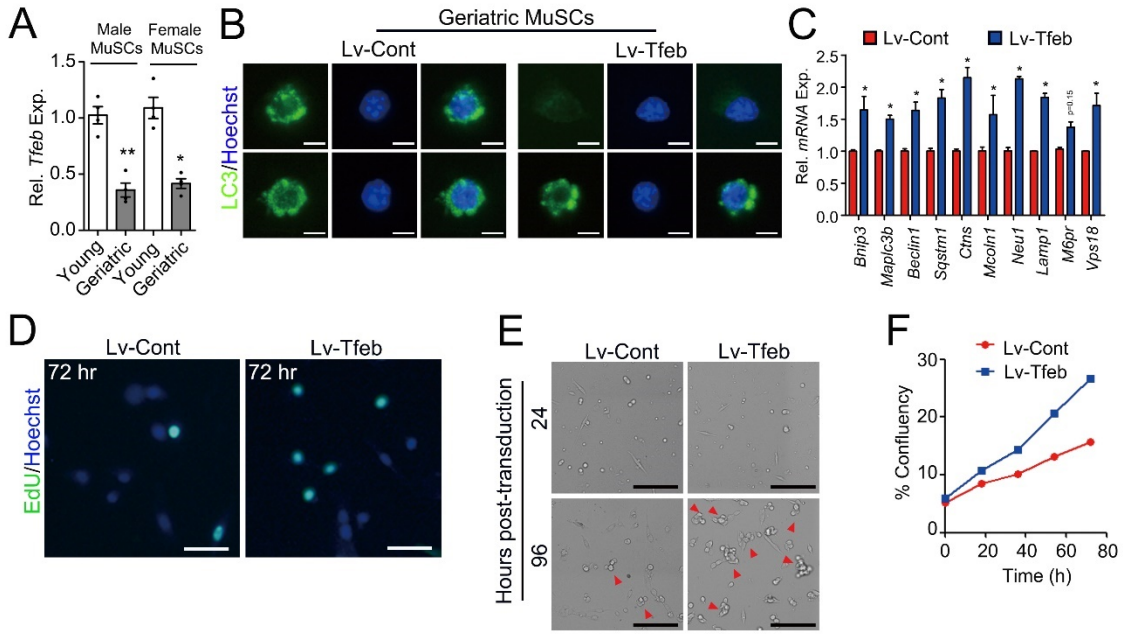

Figure S7. The restoration of Tfeb alleviates the senescence phenotypes of geriatric MuSCs.

(**A**) Relative Tfeb mRNA expression in MuSCs of young (3-month-old) and geriatric (28-month-old) mice. (**B**-**E**) Geriatric MuSCs were transduced with lentiviral vectors containing control or Tfeb [Lv-Cont-IRES-tdTomato (Lv-Cont) or Lv-Tfeb-IRES-tdTomato (Lv-Tfeb)]. MuSCs were analysed 96 hours after lentiviral transduction. Immunostaining of LC3 proteins in transduced MuSCs with or without Baf for 6 hours (B). Relative mRNA expression of autophagy and lysosomal genes in MuSCs (C). Representative images of the colony-forming assay (D) and EdU incorporation assay (E). (**F**) Representative growth curve of a live cell imaging system based cell growth assay for transduced geriatric MuSCs. Comparisons by unpaired *t*-test (A and C). Bars, mean ± s.e.m. Scales, 5 (B), 50 (D), and 100 (E) μm. Bars, mean ± s.e.m.; n = 3 (C) and 4 (A, D and E) animals per group; *P<0.05, **P<0.01.

Supporting movies

Movie S1. Representative live imaging of MuSCs freshly isolated from vehicle-treated mice 24 h after plating. Three-month-old mice were subcutaneously injected with the vehicle for 10 months and MuSCs were purified using flow cytometry for analysis.

Movie S2. Representative live imaging of MuSCs freshly isolated from Antide treated mice 24 h after plating. Three-month-old mice were subcutaneously injected with Antide for 10 months and MuSCs were purified using flow cytometry for analysis.

Movie S3. Representative live imaging of MuSCs freshly isolated from 30-month-old geriatric mice 24 h after plating.

Supporting tables

Table S1. List of autophagy-related and lysosomal genes

Table S2. Representative mass-spectrometric chromatograms and results of steroid profiling for blood samples.

References

1. Moon, J.Y., et al., *Supported liquid extraction coupled to gas chromatography-selective mass spectrometric scan modes for serum steroid profiling.* Anal Chim Acta, 2018. **1037**: p. 281-292.