Table S1. Primer list

| gene type                      | gene name       | Primer Sequence          |
|-------------------------------|-----------------|--------------------------|
| **Renin-angiotensin-system-components** |                 |                          |
| AT1                           |                 |                          |
| F                             | TCACCTGCATCATCATCTCTGG |                          |
| R                             | AGCTGGTAAGAATGATTAGG |                          |
| AT2                           |                 |                          |
| F                             | CCTGCATGAGTGTGCATAGGT |                          |
| R                             | CCAGCAGACCACTGAGCATA |                          |
| Mas                           |                 |                          |
| F                             | CCTCCCATTCTTCCAAGCTGTA |                          |
| R                             | GCCCTGGGTTGCATTTCATCTTT |                          |
| Renin                         |                 |                          |
| F                             | GCCCTCTGCCACCCAGTAA |                          |
| R                             | CAAAGCCAGACACAAATGGCCCC |                          |
| Angiotensinogen               |                 |                          |
| F                             | ACCCCCGAGTGGGAGAGTTTC |                          |
| R                             | GCCAGGCTGCTGGACAGACG |                          |
| ACE                           |                 |                          |
| F                             | TCATCATCCAGTTCCAGTCCA |                          |
| R                             | CGGTGAGCAGCCATTCTGT |                          |
| ACE2                          |                 |                          |
| F                             | CTACAGGCCCTTCAGCAAGAG |                          |
| R                             | AATGGTGCTCATGGGTCTTTCA |                          |
| **Cell cycle arrest and senescence** |                 |                          |
| p16                           |                 |                          |
| F                             | CCGCTGCAGACAGACTGG |                          |
| R                             | CATCATCATCACCTGAAATCG |                          |
| p19                           |                 |                          |
| F                             | AAGAGAGGTTTTTCTTGGTG |                          |
| R                             | CATCATCATCACCTGGTCC |                          |
| p21                           |                 |                          |
| F                             | GACAAGAGGCCAGTACTTCCT |                          |
| R                             | CAATCTGCGCTTGGAGTGATA |                          |
| p53                           |                 |                          |
| F                             | CCCCTGTCTATTTTGTGCCCTT |                          |
| R                             | GGGAGGAGAGTACGTGCACATAA |                          |
| gene type | gene name | Primer Sequence |
|-----------|-----------|-----------------|
| SASP      | PAI-1     | F: TCAGAGCAACAAGTTCAACTACACTGAG  
                      | R: CCCACTGTCAAGGCTCCATCACTTGCCCCCA |
|           | IGFBP2    | F: GGGTGCCAACACACCTCAG  
                      | R: AGGTTGTACCGGCCATGC |
|           | MMP13     | F: CTTCTACCCATTTTGATGGACCTT  
                      | R: AAGCTCATGGGCAGCAAACA |
|           | IL-6      | F: CTACCCCAATTTCACAATGCT  
                      | R: ACCACAGTGAGGAATGTCCA |
|           | TNF-α     | F: CCCTCACACTCAGATCATCTTCT  
                      | R: GCTACGACGTGGGCTACAG |
|           | MCP-1     | F: TGTAAGTTTCTGTACATCTTTGG  
                      | R: ACTCATTCACCAGCAAGATG |
| Muscle specific gene signatures of ageing | MuRF-1 | F: GTGAAGTTGCCCCCCTTACAA  
                      | R: TGGAGATGCAATTGCTCAGT |
|           | Atrogin-1 | F: CCATCAGGAGAAGTGGATCTATGTT  
                      | R: GCTTCCCCAAAAGTGCAAGTA |
|           | Myostatin | F: CATCTTGTCACCAAGCAAAA  
                      | R: GGGAGACATTTTGTGCGAGT |
| Regulatory factor for mitochondrial fusion | Mfn1 | F: AGCCCAACATCTTCATTCTGAA  
                      | R: CTTACAACCTTGAGCTTCTTACCA |
|           | Mfn2      | F: CATCAGTTACCCGGCTCTAACT  
                      | R: GAGCCTCGACCTTTCTTGTCAA |
|           | DRP1      | F: GTTCCACGCCAACAGAAATAC  
                      | R: CCTACCCCCCTGAATGAAGT |
| gene type         | gene name | Primer Sequence         |
|-------------------|-----------|-------------------------|
| Fibrosis          | αSMA      | F GTCCCAGACATCAGGGAGTAA |
|                   |           | R TCGGATACTTCAGCGTCAGGA |
|                   | TGF-β1    | F CCCCTGGAAAGGGCTCAACAC |
|                   |           | R TCCAACCCAGGTCTTCTAAAGTC |
|                   | COL1A1    | F GCCGCAAAGAGTCTACA     |
|                   |           | R CGGGTTTCCACGTCTCA     |
|                   | CTGF      | F TCCACCCGAGTTACCAA     |
|                   |           | R TTAGGTGTCCGGATGC      |
| House keeping gene| GAPDH     | F TTGTGATGGGTGTGAAACCACGAGA |
|                   |           | R CATGAGCCCTTCCACACAATGCCAAA |
|                   | 18S       | F CGATCCGAGGGCCTCACTA   |
|                   |           | R AGTCCCTGGCCCTTGTA     |
Histological analysis of the tibialis anterior muscle in mice.

Representative H&E-stained sections of the tibialis anterior muscle in each group of mice (a). Average value and distribution of CSA. The frequency of myofibers was adjusted as a percentage of the total number of fibers and displayed as a histogram (b).

Approximate number of muscle fibers in the tibialis anterior muscle, as estimated by dividing the muscle area by the CSA of a single muscle fiber in each mouse (c).

Central nucleated fibers (CNFs) expressed as a percentage of total myofibers (d).

Leucocyte infiltration determined as infiltrated nucleus expressed as a percentage of myofibers (e).

Differences were analyzed with a one-way ANOVA using a Bonferroni post-hoc test.

*p < 0.05; n.s., not significant

Scale bars: 200 μm. Circles indicate the centrally located nuclei. Arrows indicate the infiltrated leucocytes. WT, Wild-type.
Histological analysis of the vastus muscle in mice.
Representative H&E-stained sections of the vastus muscle in each group of mice (a). Average value and distribution of CSA. The frequency of myofibers was adjusted as a percentage of the total number of fibers and displayed as a histogram (b). Approximate number of muscle fibers in the vastus muscle, as estimated by dividing the muscle area by the CSA of a single muscle fiber in each mouse (c). Central nucleated fibers (CNFs) expressed as a percentage of total myofibers (d). Leucocyte infiltration determined as infiltrated nucleus expressed as a percentage of myofibers (e). Differences were analyzed with a student’s t-test.
*p < 0.05; n.s., not significant
Scale bars: 200 μm. Circles indicate the centrally located nuclei. Arrows indicate the infiltrated leucocytes.
Histological analysis of the vastus muscle in mice.

Representative Masson’s tricrome-stained sections of the vastus muscle in each group of mice (a).

Percentage of fibrosis area (b) and number of vessels (c) in muscle fibers.

Differences were analyzed with a student’s t-test.

*p < 0.05; n.s., not significant

Scale bars: 200 μm.
Histological analysis of the tibialis anterior muscle in mice.
Representative H&E-stained sections of the tibialis anterior muscle in each group of mice (a).
Average value and distribution of CSA. The frequency of myofibers was adjusted as a percentage of the total number of fibers and displayed as a histogram (b).
Approximate number of muscle fibers in the vastus muscle, as estimated by dividing the muscle area by the CSA of a single muscle fiber in each mouse (c).
Central nucleated fibers (CNFs) expressed as a percentage of total myofibers (d).
Leucocyte infiltration determined as infiltrated nucleus expressed as a percentage of myofibers (e).
Differences were analyzed with a student’s t-test.
*p < 0.05; n.s., not significant
Scale bars: 200 μm. Circles indicate the centrally located nuclei. Arrows indicate the infiltrated leucocytes.
**Fig. S5**

(a) Representative skin sections visualized by H&E staining (a).

(b) Quantification of thickness in dermis and epidermis layers (b) and fat layers (c). Differences were analyzed with a one-way ANOVA using a Bonferroni post-hoc test.

**Histological analysis of the skin in mice.**
Representative skin sections visualized by H&E staining (a).
Quantification of thickness in dermis and epidermis layers (b) and fat layers (c).
Differences were analyzed with a one-way ANOVA using a Bonferroni post-hoc test. n.s., not significant

WT, Wild-type; KO, knockout
Fig. S6

(a)

Tibialis anterior muscle

|        | AT1    | AT2    | Mas    | Renin  |
|--------|--------|--------|--------|--------|
|        | ![](image) | ![](image) | ![](image) | ![](image) |

Angiotensinogen | ACE | ACE2 | WT | AT2 KO | AT2/Mas KO

Gastrocnemius muscle

|        | AT1    | AT2    | Mas    | Renin  |
|--------|--------|--------|--------|--------|
|        | ![](image) | ![](image) | ![](image) | ![](image) |

Angiotensinogen | ACE | ACE2 | WT | AT2 KO | AT2/Mas KO

○ WT ○ AT2 KO ○ AT2/Mas KO
(b)  

**Tibialis anterior muscle**

- **$p16$**
- **$p19$**
- **$p21$**
- **$p53$**
- **PAI-1**

**IGFBP2**

**MMP13**

**IL-6**

**TNF-α**

**MCP-1**

**Gastrocnemius muscle**

- **$p16$**
- **$p19$**
- **$p21$**
- **$p53$**
- **PAI-1**

**IGFBP2**

**MMP13**

**IL-6**

**TNF-α**

**MCP-1**

○ WT  ○ AT2 KO  ● AT2/Mas KO
(c)

Tibialis anterior muscle

| Gene  | MuRF-1 | Atrogin-1 | Myostatin | Mfn1 | Mfn2 |
|-------|---------|-----------|-----------|------|------|
|       | ![Graph](image1) | ![Graph](image2) | ![Graph](image3) | ![Graph](image4) | ![Graph](image5) |

| Gene  | DRP1 | α-SMA | TGF-β1 | Col1A1 | CTGF |
|-------|------|-------|--------|--------|------|
|       | ![Graph](image6) | ![Graph](image7) | ![Graph](image8) | ![Graph](image9) | ![Graph](image10) |

Gastrocnemius muscle

| Gene  | MuRF-1 | Atrogin-1 | Myostatin | Mfn1 | Mfn2 |
|-------|---------|-----------|-----------|------|------|
|       | ![Graph](image11) | ![Graph](image12) | ![Graph](image13) | ![Graph](image14) | ![Graph](image15) |

| Gene  | DRP1 | α-SMA | TGF-β1 | Col1A1 | CTGF |
|-------|------|-------|--------|--------|------|
|       | ![Graph](image16) | ![Graph](image17) | ![Graph](image18) | ![Graph](image19) | ![Graph](image20) |

*WT*  	*AT2 KO*  	*AT2/Mas KO*
RT-qPCR analysis of age-induced changes in genes associated with RAS, aging, inflammation, mitochondrial function, and fibrosis in the tibialis-anterior muscle and gastrocnemius muscle in mice.
Quantitative estimation of the expression levels of genes associated with RAS (a), aging and pro-inflammatory senescence-associated secretory phenotype (b), and muscle-specific gene markers of senescence, mitochondrial function, and fibrosis (c), relative to GAPDH expression in the tibialis-anterior muscle and gastrocnemius muscle. Differences were analyzed with a one-way ANOVA using the Bonferroni post-hoc test.
*, p < 0.05
No significant difference was observed among the groups otherwise indicated.
WT, Wild-type; KO, knockout
RT-qPCR analysis of genes associated with RAS, aging, inflammation, and fibrosis in tibialis anterior muscles, heart, and adipose tissues in old mice.

Expression of genes associated with RAS (a) and aging and proinflammatory senescence-associated secretory phenotype (b) relative to GAPDH expression in liver anterior muscles, heart, and adipose tissues.

Differences were analyzed with a one-way ANOVA using a Bonferroni post-hoc test.

*, p < 0.05

No significant difference was observed among the groups otherwise indicated.

WT, Wild-type, KO, knockout
Relative abundance of genes associated with RAS in muscles, liver, heart, and adipose tissue in 24 month-old wildtype mice.
Quantitative estimation of the expression levels of genes associated with RAS.
Comparison of the expression levels of genes associated with RAS across the organs.
Y-axis represents log-transformed ΔΔCt of each gene normalized to 18S.
VM, vastus muscle; TA, tibialis anterior muscle; GM, gastrocnemius muscle
Aging associated changes in the expression of the renin-angiotensin system (RAS) components in tibialis anterior muscles in mice.
Expression of genes associated with RAS in tibialis anterior muscles of young (3-months old) and aged (24-months old) wildtype mice, relative to GAPDH expression. No significant difference was observed between the age-groups analyzed by a Student's t-test.
Succinate dehydrogenase (SDH) activities of the vastus muscles

Representative SDH-stained sections of the vastus muscle in each group of mice (a). Average value and distribution of SDH intensity. Differences were analyzed with a one-way ANOVA using a Bonferroni post-hoc test.

n.s., not significant

Scale bars: 200 μm.