*Supplementary material*

# Resveratrol counteracts bone loss via mitofilin-mediated osteogenic improvement of mesenchymal stem cells in senescence-accelerated mice

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**Supplementary material:** 5 figures with their legends and 1 table.

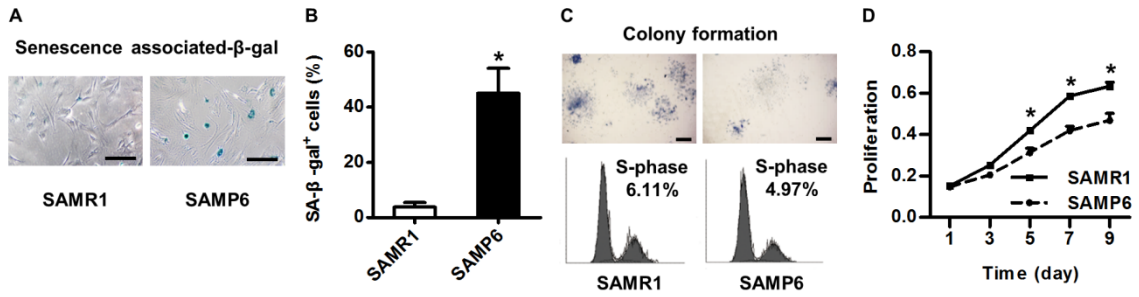


**Figure S1. Cell senescence of BMMSCs from SAMP6 mice. (A, B)** Representative images of SA-β-gal staining **(A)** with quantification of positively stained cells **(B)** in BMMSCs from SAMR1 and SAMP6 mice at 4 months of age. Bars: 200 μm. **(C)** Representative images of cell colonies with flow cytometric analysis of cell cycle in BMMSCs from SAMR1 and SAMP6 mice at 4 months of age. Bars: 200 μm. **(D)** MTT analysis of cell proliferation in BMMSCs from SAMR1 and SAMP6 mice at 4 months of age. $n = 3$ per group. Data represent mean ± SD. $^*P < 0.05$. Data were analyzed using the two-tailed Student's *t* test.

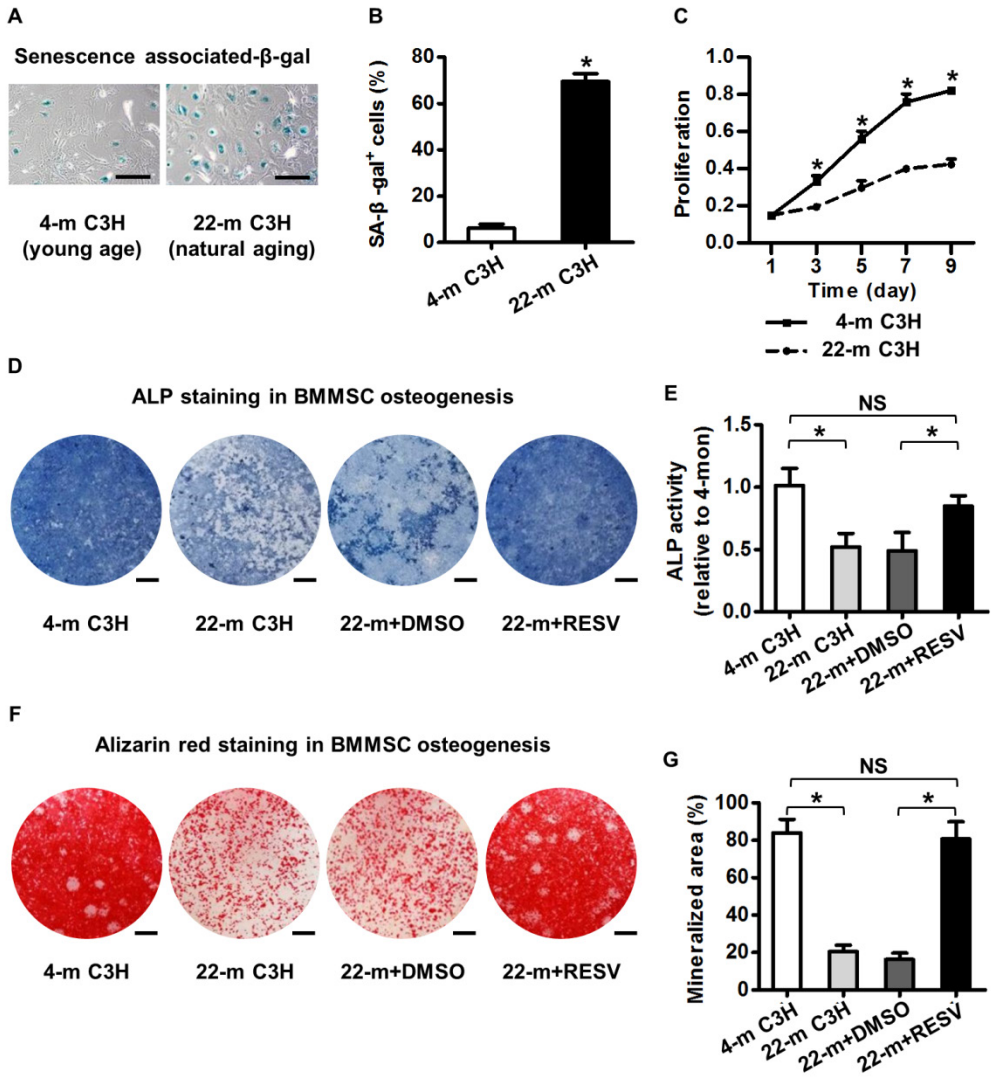


**Figure S2. Resveratrol rescues osteogenic decline of BMMSCs derived from natural aging mice. (A, B)** Representative images of SA-β-gal staining **(A)** with quantification of positively stained cells **(B)** in BMMSCs from C3H mice at 4 months and 22 months of age. Bars: 200 μm. **(C)** MTT analysis of cell proliferation in BMMSCs from C3H mice at 4 months and 22 months of age. **(D, E)** Representative images of ALP staining **(D)** with quantification of ALP activity **(E)** in osteogenic differentiation of BMMSCs. Bars: 5 mm. **(F, G)** Representative images of alizarin red staining **(F)** with quantification of mineralization **(G)** in osteogenic differentiation of BMMSCs. Bars: 5 mm. BMMSCs from C3H mice at 22 months of age were treated with either resveratrol (10 μM) or the DMSO (0.001%) solvent control. $n = 3$ per group. Data represent mean ± SD. *$P <$

0.05; NS, not significant ($P > 0.05$). Data were analyzed using the two-tailed Student's *t* test **(B, C)** or ANOVA followed by Newman-Keuls post-hoc tests **(E, G)**.

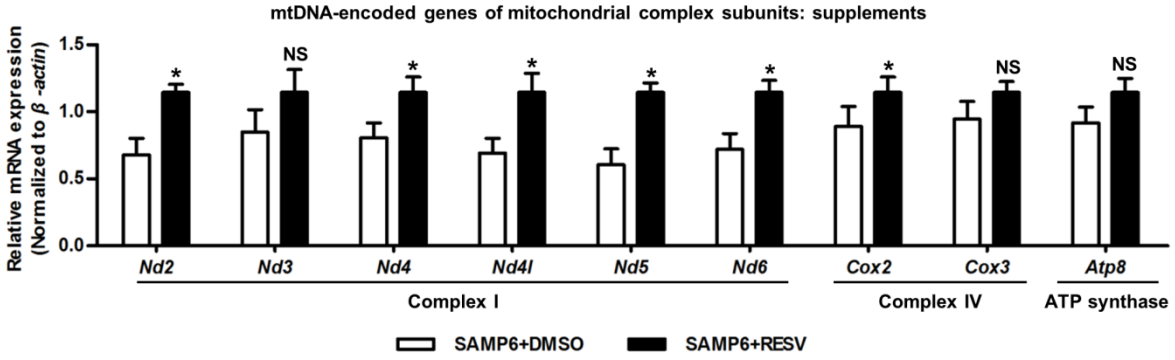


**Figure S3. General effects of resveratrol on mtDNA-encoded mitochondrial complex subunits in SAMP6 BMMSCs.** qRT-PCR analysis of mRNA expression levels of mtDNA-encoded mitochondrial complex subunits in senescent BMMSCs (related to **Figure 3H** as supplements). BMMSCs from SAMP6 mice at 4 months of age were treated with either resveratrol (10 μM) or the DMSO (0.001%) solvent control. $n$ = 3 per group. Data represent mean ± SD. *$P$ < 0.05; NS, not significant ($P$ > 0.05). Data were analyzed using the two-tailed Student's $t$ test.

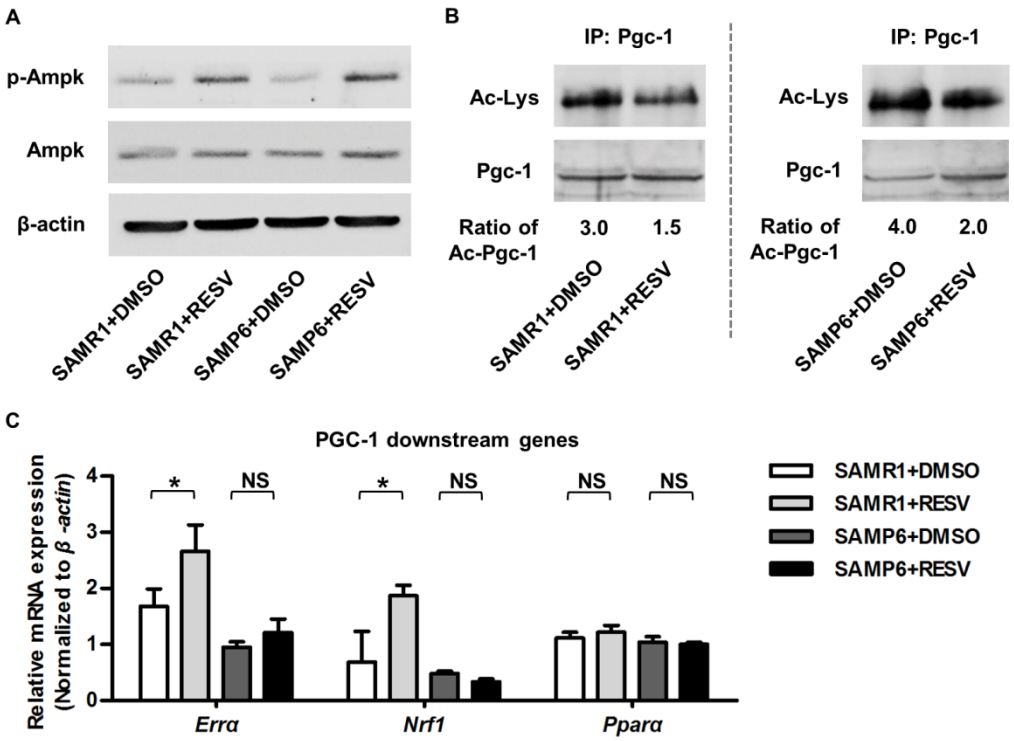


**Figure S4. Effects of resveratrol on Ampk and Pgc-1 pathways in SAMR1 and SAMP6 BMMSCs. (A)** Western blot analysis of total and phosphorylated protein expression levels of Ampk in BMMSCs. **(B)** Western blot analysis of total and acetylated protein expression levels of Pgc-1 in BMMSCs. Acetylated Pgc-1 was immunoblotted with an anti-acetylated lysine antibody after immunoprecipitation of Pgc-1 from nuclear extracts. **(C)** qRT-PCR analysis of mRNA expression levels of Pgc-1 downstream targets in BMMSCs. BMMSCs from 4-month-old SAMR1 and SAMP6 mice were treated with either resveratrol (10 μM) or the DMSO (0.001%) solvent control. $n = 3$ per group. Data represent mean ± SD. $^*P < 0.05$; NS, not significant ($P > 0.05$). Data were analyzed using ANOVA followed by Newman-Keuls post-hoc tests.

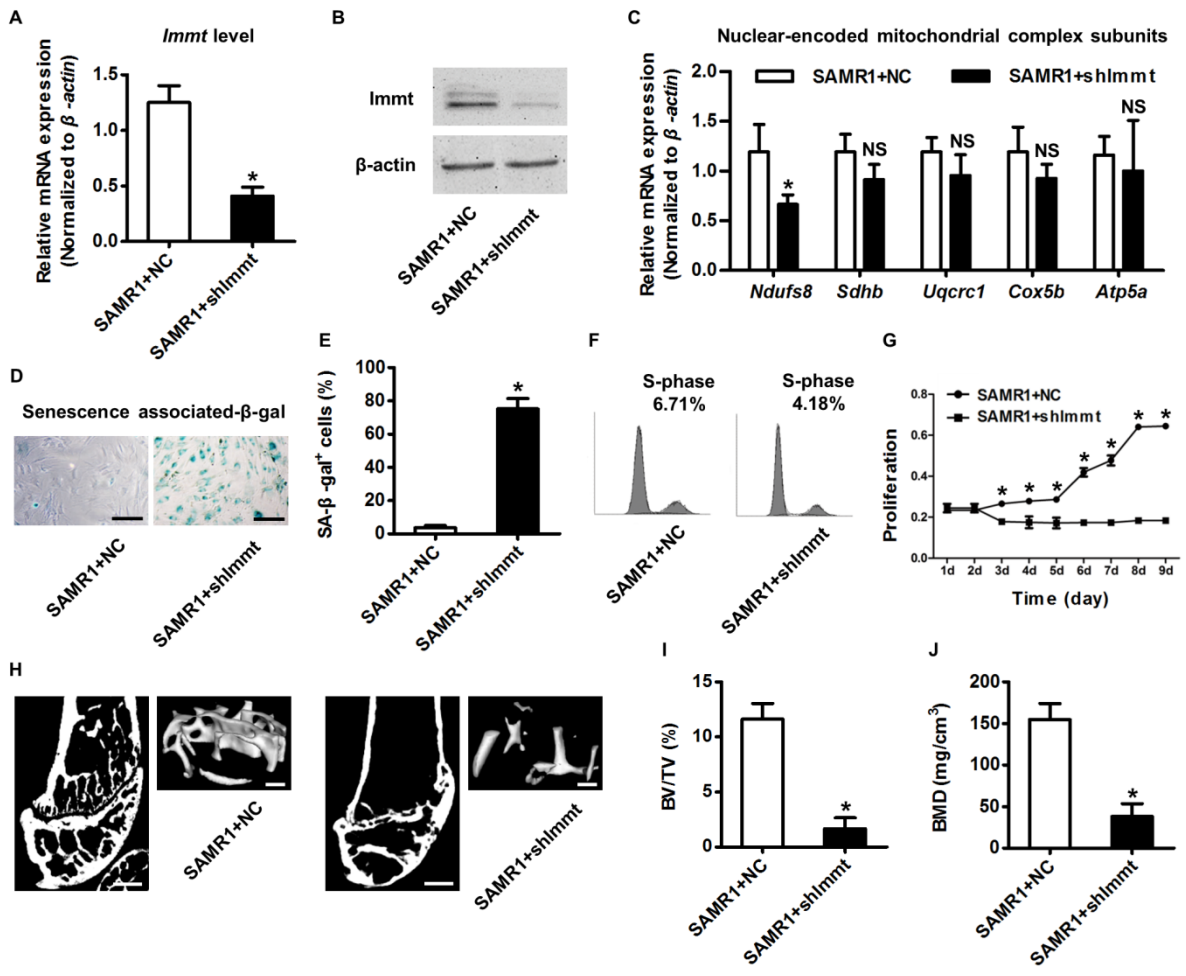


**Figure S5. Knockdown of Mitofilin mimics senescence of BMMSCs and induces bone loss. (A, B)** qRT-PCR analysis of mRNA expression **(A)** and western blot analysis of protein expression **(B)** levels of *Immt* (Mitofilin) after shRNA-mediated knockdown in BMMSCs from SAMR1 mice at 4 months of age. **(C)** qRT-PCR analysis of mRNA expression levels of nuclear-encoded mitochondrial complex subunit genes in BMMSCs (related to **Figure 5J**). **(D, E)** Representative images of SA-β-gal staining **(D)** with quantification of positively stained cells **(E)** in BMMSCs. Bars: 200 μm. **(F)** Flow cytometric analysis of cell cycle in BMMSCs. **(G)** MTT analysis of cell proliferation in BMMSCs. BMMSCs from SAMR1 mice at 4 months of age were transfected with either the shRNA for Immt (Mitofilin) or the negative control (a scrambled sequence, NC) by a lentiviral vector. **(H-J)** Representative 2D section and 3D reconstruction micro-CT images **(H)** and quantitative analysis of trabecular

bone volume **(I)** and bone mineral density **(J)** in distal femora. Bars: 500 μm. SAMR1 mice at 4 months of age were treated intra-bone marrow with either the shRNA for Immt (Mitofilin) or the negative control (a scrambled sequence, NC) in a lentiviral vector solution and were sacrificed at 6-month-old. $n = 3$ per group. Data represent mean ± SD. *$P < 0.05$; NS, not significant ($P > 0.05$). Data were analyzed using the two-tailed Student's *t* test.

**Table S1. Primer sequences for mouse genes detected in the present study.**

| Gene | Primer sequences |
|---|---|
| *β-actin* | Forward: 5'-CATCCGTAAAGACCTCTATGCCAAC-3' |
| | Reverse: 5'-ATGGAGCCACCGATCCACA-3' |
| *Alp* | Forward: 5'-CCTTGTAGCCAGGCCCATTG-3' |
| | Reverse: 5'-GGACCATTCCCACGTCTTCAC-3' |
| *Atp5a* | Forward: 5'-CATTGGTGATGGTATTGCGC-3' |
| | Reverse: 5'-TCCCAAACACGACAACTCC-3' |
| *Atp6* | Forward: 5'-TCCCAATCGTTGTAGCCATC-3' |
| | Reverse: 5'-TGTTGGAAAGAATGGAGTCGG-3' |
| *Atp8* | Forward: 5'-GCCACAACTAGATACATCAACATG-3' |
| | Reverse: 5'-TGGTTGTTAGTGATTTTGGTGAAG-3' |
| *Cox1* | Forward: 5'-CCCAGATATAGCATTCCCACG-3' |
| | Reverse: 5'-ACTGTTCATCCTGTTCCTGC-3' |
| *Cox2* | Forward: 5'-AGTTGATAACCGAGTCGTTCTG-3' |
| | Reverse: 5'-CTGTTGCTTGATTTAGTCGGC-3' |
| *Cox3* | Forward: 5'-CGTGAAGGAACCTACCAAGG-3' |
| | Reverse: 5'-CGCTCAGAAGAATCCTGCAA-3' |
| *Cox5b* | Forward: 5'-ACCCTAATCTAGTCCCGTCC-3' |
| | Reverse: 5'-CAGCCAAAACCAGATGACAG-3' |
| *Cytb* | Forward: 5'-CCCACCCCATATTAAACCCG-3' |
| | Reverse: 5'-GAGGTATGAAGGAAAGGTATAAGGG-3' |

| | |
|---|---|
| *Drp1* | Forward: 5’-ACTGGCCCCCGTCCTGCTTTAT-3’ |
| | Reverse: 5’-ATGGACCAGCTCCACACACCGT-3’ |
| *Errα* | Forward: 5’-GGGGAGCATCGAGTACAGC-3’ |
| | Reverse: 5’-AGACGCACACCCTCCTTGA-3’ |
| *Immt* | Forward: 5’-AAGGTCCAAGCAGCTCAGTCT-3’ |
| | Reverse: 5’-TGTTTCTCCAAGGCTAACGTGA-3’ |
| *Mfn1* | Forward: 5’-TCGGTTTTCCCTGGGCTGGT-3’ |
| | Reverse: 5’-TCGACGTGAGGGACGCCAAT-3’ |
| *Mfn2* | Forward: 5’-AAGCCCAGGGCATGCCAGAA-3’ |
| | Reverse: 5’-TGAGCTGCGATGTGCAGGGA-3’ |
| *Nd1* | Forward: 5’-TGCACCTACCCTATCACTCA-3’ |
| | Reverse: 5’-GGCTCATCCTGATCATAGAATGG-3’ |
| *Nd2* | Forward: 5’-ATACTAGCAATTACTTCTATTTTCATAGGG-3’ |
| | Reverse: 5’-GAGGGATGGGTTGTAAGGAAG-3’ |
| *Nd3* | Forward: 5’-AAGCAAATCCATATGAATGCGG-3’ |
| | Reverse: 5’-GCTCATGGTAGTGGAAGTAGAAG-3’ |
| *Nd4* | Forward: 5’-CATCACTCCTATTCTGCCTAGC-3’ |
| | Reverse: 5’-CCAACTCCATAAGCTCCATACC-3’ |
| *Nd4l* | Forward: 5’-CCAACTCCATAAGCTCCATACC-3’ |
| | Reverse: 5’-GATTTTGGACGTAATCTGTTCCG-3’ |
| *Nd5* | Forward: 5’-ACGAAAATGACCCAGACCTC-3’ |
| | Reverse: 5’-GAGATGACAAATCCTGCAAAGATG-3’ |

| | |
|---|---|
| *Nd6* | Forward: 5’-TGTTGGAGTTATGTTGGAAGGAG-3’ |
| | Reverse: 5’-CAAAGATCACCCAGCTACTACC-3’ |
| *Ndufs8* | Forward: 5’-GTTCATAGGGTCAGAGGTCAAG-3’ |
| | Reverse: 5’-TCCATTAAGATGTCCTGTGCG-3’ |
| *Nrf1* | Forward: 5’-AATGTCCGCAGTGATGTCC-3’ |
| | Reverse: 5’-GCCTGAGTTTGTGTTTGCTG-3’ |
| *Ocn* | Forward: 5’-TGACAAAGCCTTCATGTCCAA-3’ |
| | Reverse: 5’-GCGCCGGAGTCTGTTCACTA-3’ |
| *Opa1* | Forward: 5’-ATAACTACCCGCGCCTGCGA-3’ |
| | Reverse: 5’-TGCTTTGGCGTGACCTGGCT-3’ |
| *Osx* | Forward: 5’-GGGCGTTCTACCTGCGACTG-3’ |
| | Reverse: 5’-CGAAGCCTTGCCGTACACCT-3’ |
| *Phb* | Forward: 5’-TGGCAGAAGAAAGGCAGGGCA-3’ |
| | Reverse: 5’-TCACACACACCTGCTTCCGCT-3’ |
| *Pink1* | Forward: 5’-AAGCGCGTGTCTGACCCACT-3’ |
| | Reverse: 5’-ACACAGCGGCATTGCAACCCT-3’ |
| *Pparα* | Forward: 5’-AGGAAGCCGTTCTGTGACAT-3’ |
| | Reverse: 5’-GCCTGAGTTTGTGTTTGCTG-3’ |
| *Runx2* | Forward: 5’-CCGCACGACAACCGCACCAT-3’ |
| | Reverse: 5’-CGCTCCGGCCCACAATCTC-3’ |
| *Sdhb* | Forward: 5’-ACCCCTTCTCTGTCTACCG-3’ |
| | Reverse: 5’-TCCATTAAGATGTCCTGTGCG-3’ |

| | | |
|---|---|---|
| *Uqcrc1* | Forward: 5’-ATCAAGGCACTGTCCAAGG-3’ | |
| | Reverse: 5’-TCATTTTCCTGCATCTCCCG-3’ | |