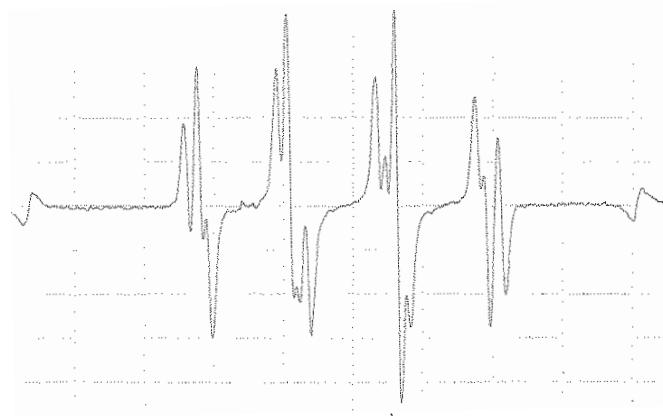
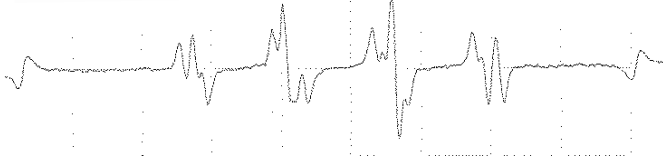


Supplemental
Figure

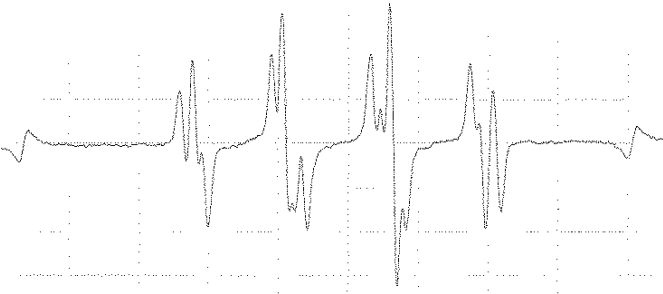
Without sample



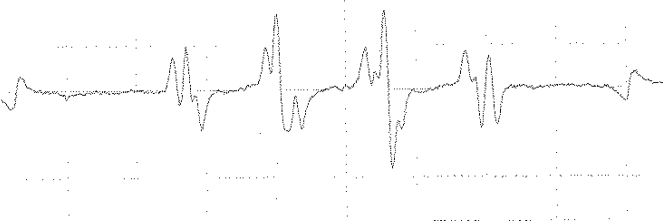
Young mice



Old mice



Old mice
+MitoTEMPO

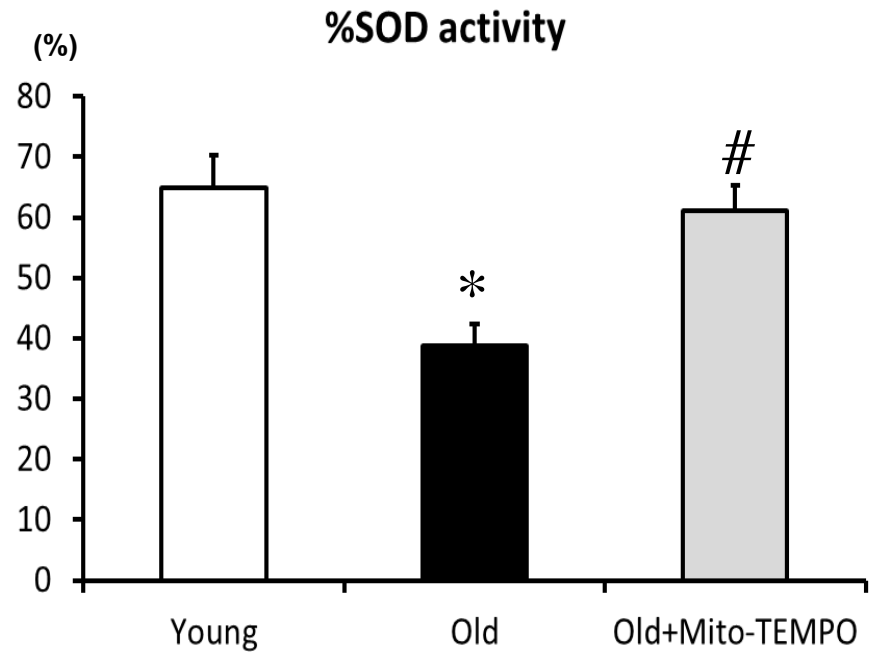


SOD standard



Mn²⁺

Mn²⁺



Supplemental figure legend

Superoxide anion radical ($O_2^{\cdot -}$) scavenging activity in mitochondria

An ESR spectrometer (JES-FR 30; JEOL, Tokyo, Japan) equipped with manganese oxide (MnO) as an internal standard was used for measuring $O_2^{\cdot -}$ scavenging activities. The conditions for ESR were as follows: magnetic field: 335 ± 5 mT; power: 4 mW; modulation width: 0.079 mT; modulation amplitude: 1×0.1 mT; response time: 0.1 sec; amplitude: 1×200 ; sweep width: 5.000 mT; and sweep time: 2 min. The method for spin trapping of the $O_2^{\cdot -}$ radical was based on previous studies.¹ To prepare the solution, a mixture was added containing 50 μ l of 5 mM hypoxanthine (Sigma Chemical), 15 μ l of 5, 5'-dimethyl-1-pyrroline- N - oxide (DMPO, Labotec Co. Tokyo, Japan), 50 μ l of sample (n=10, each) or superoxide dismutase (SOD) -bovine erythrocytes (Calbiochem, Inc., La Jolla, CA), 50 μ l of 0.4 units/ml xanthine oxidase (XOD) (Roche K. K., Tokyo, Japan). After mixing well, the reaction mixture was transferred onto a quartz flat cell, and monitoring of the ESR spectrum was started exactly 1 min after the addition of XOD. All solutions were prepared with 0.1 M potassium phosphate buffer (pH 7.4). %SOD activity in mitochondria was shown as [(DMPO- $O_2^{\cdot -}$ signal before administration of sample - DMPO- $O_2^{\cdot -}$ signal after administration of sample/DMPO- $O_2^{\cdot -}$ signal before administration of sample) x 100 (%)]. Values were expressed as the means \pm S.E.M. n=12 each, * p <0.01 vs. young mice, # p <0.01 vs. old mice.

Typical ESR spectra of DMPO- $O_2^{\cdot -}$ at various ages of mitochondria. The attenuation of the signal by SOD shows the component dependent on $O_2^{\cdot -}$ (Left panel). SOD activity was decreased in mitochondria from old mice compared to that from young mice. Treatment of MitoTEMPO improved the SOD activity in old mice (Right panel).

1. Machii H, Saitoh S, Kaneshiro T, Takeishi Y. Aging impairs myocardium-induced dilation in coronary arterioles: Role of hydrogen peroxide and angiotensin. *Mech Age Develop.* 2010; 131, 710-717.