Supplementary material

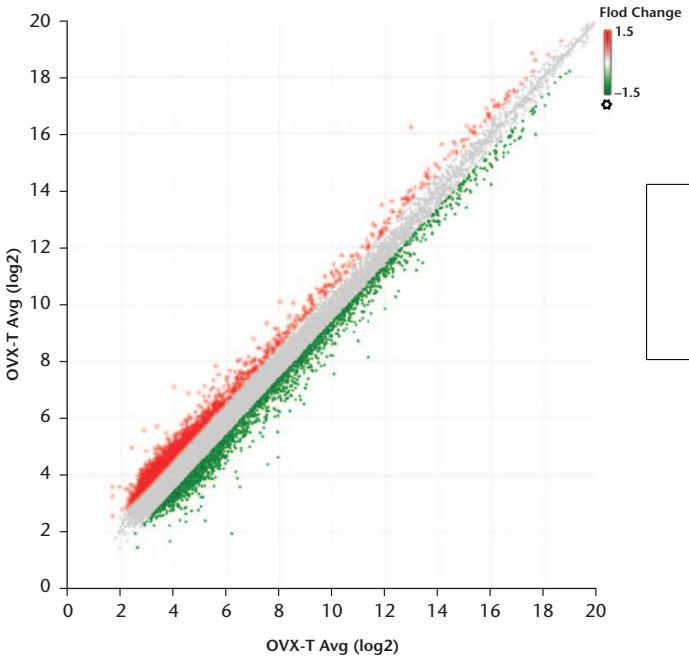

Fig. aa

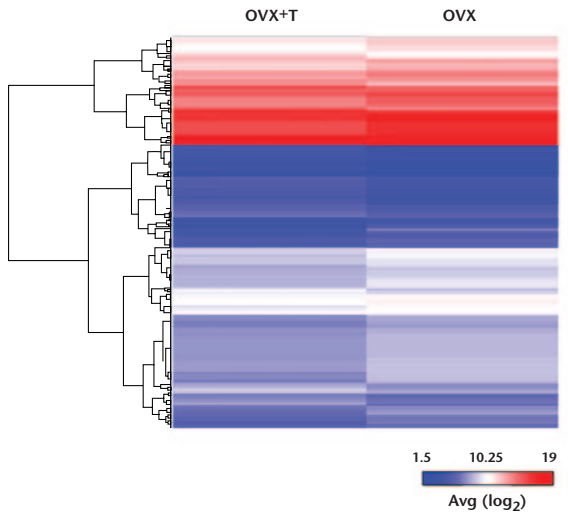

Fig. ab

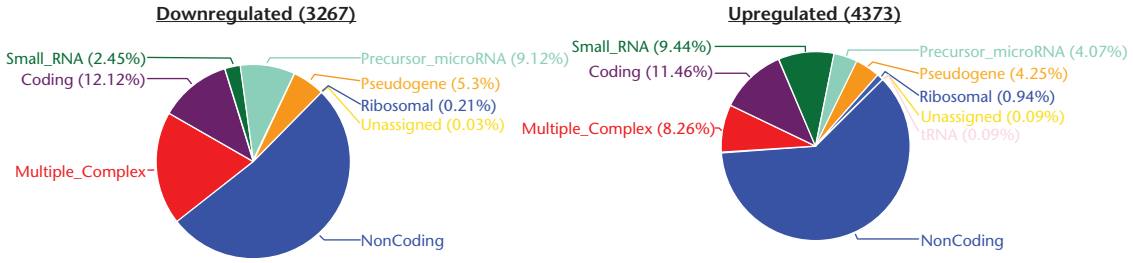

Fig. ac

Transcriptomic comparison of osteoblasts (OB) between ovariectomized mice that undertook exercise (OVX+T) and those that did not (OVX). aa) Hierarchical clustering was employed to grouping gene expression of OB in the OVX+T and OVX mouse. ab) Scatter plot was applied to representing the different expression value of a gene in the OVX+T and OVX group. Red dots indicate genes that have been upregulated in the OVX+T group compared with the OVX group; green dots indicate genes that have been downregulated in the OVX+T group compared with OVX group. ac) The relative percentage of different type genes upregulated or downregulated (>1.5 or <-1.5-fold change compared with OVX group) was presented in a pie chart.

Supplementary Methods

Mouse strain and training protocol. Irregular cycling oestrogen level begins at 8- to 12-month-old female C57BL/6 mice. Thereafter, the oestrogen level declines by 45% to 80%, but persists at a detectable level.[1,2] Uterine weight, a physiological indicator of oestrogen, keeps on normal levels up to at least 31 months.[3] So, there is no true menopause in mice. Meanwhile, ageing is associated with elevated levels of inflammatory cytokines. This is caused by innate cells such as macrophages, a process known as inflamm-ageing.[4] Aged macrophages have increased nitric oxide production under the resting condition and higher surface density of TLR4, leading to a faster and enhanced inflammatory response.[5,6] The age-induced inflammatory cytokines will be beneficial for OC formation. To minimize these influences, we chose the adult ovariectomized mice for our study.

Chen et al[7] reported that medium-intensity treadmill exercise (speed: 12 m/mime to 18 m/mime; time: 20 mins to 50 mins; frequency: six days/week; duration: nine weeks) was more effective on the increase of BMD and bone strength than a low-intensity exercise in the senile mice. Based on the protocol, we trained ovariectomized mice with modified medium-intensity treadmill (speed: 10 m/mime; time: 60 mins; frequency: five days/week; duration: eight weeks) in the present study.

Table i. WikiPathway analysis of differentially expressed genes

Pathway	Downregulated genes	Upregulated genes
IL-1 signalling pathway	Il1b, Il1rn, Il1r1	
IL-6 signalling pathway	Il6, Il6ra, Il6st, Btk, Jak1, Crebbp, Rb1	
IL-7 signalling pathway	Pik3r1, Il7r, Jak1, Irs2, Rb1, Cblb, Cbl	
TNF-alpha NF-kB signalling pathway	Rnf216, Casp8ap2, Tnfrsf1b, Nfkbia, Psmd, Papola, Glg1, Birc2, Crebbp, Tnfrsf11a	
Inflammatory response pathway	Col3a1, Lamc1, Tnfrsf1b	
TGF-beta signalling pathway	Tgfbr3, Jak1, Zeb2, Crebbp, Skil, Tgfbr3, Prkar2a, Skil, Anapc1, Ctcf, Map2k3, Crebbp, Kpnb1, Rb1, Cdk6	Serpine1

Table ii. Primer sequence

Gene	Sequence
DMP-1-forward	5′-GCTGAGGTTTTGACCTTGTGG-3′
DMP-1-reverse	5′-CAGCCAAATCACCCGTCCT -3′
SLc13A5-forward	5′-CATGAGACACAATCATATCACAGAT-3′
SLc13A5-reverse	5′- TTGGGCGACTTTCCAATCCA- 3′
IBSP-forward	5′- ACAATCCGTGCCACTCACTC -3′
IBSP-reverse	5′- CCGGTACTTAAAGACCCCGTT -3′
Acp5-forward	5′- GTGATCACCGCTTTTGGTCC -3′
Acp5-reverse	5′- ACCACCCATGAATCCATCCTG -3′
THBS4-forward	5′-CAGCCAGTCCTGACAGATCC-3′
THBS4-reverse	5′-TAGCGGAGGATGGCTTTGTT- 3′
MMP9-forward	5′-CAGCCGACTTTTGTGGTCTTC- 3′
MMP9-reverse	5′- GTACAAGTATGCCTCTGCCA - 3′
PI15-forward	5′- TGCAACTATGCTCCAAAGGGT - 3′
PI15-reverse	5′- ACGATGAACATGGCACTCCA - 3′
LPL-forward	5′- AGAGAGGACTCGGAGACGTG - 3′
LPL-reverse	5′- GGAGTTGCACCTGTATGCCT - 3′
ALP-forward	5′-CTTGCTGGTGGAAGGAGGCAGG-3′
ALP-reverse	5′-CACGTCTTCTCCACCGTGGGTC-3′
Col I-forward	5′-ATCTCCTGGTGCTGATGGAC-3′
Col I-reverse	5′-ACCTTGTTTGCCAGGTTCAC-3′
GAPDH-forward	5′-CGACTTCAACAGCAACTCCCACTCTTCC-3′
GAPDH-reverse	5′- TGGGTGGTCCAGGGTTTCTTACTCCTT-3′

References

1. **Mobbs CV, Cheyney D, Sinha YN, Finch CE.** Age-correlated and ovary-dependent changes in relationships between plasma estradiol and luteinizing hormone, prolactin, and growth hormone in female C57BL/6J mice. *Endocrinology* 1985;116:813-820.
2. **Nelson JF, Felicio LS, Osterburg HH, Finch CE.** Differential contributions of ovarian and extraovarian factors to age-related reductions in plasma estradiol and progesterone during the estrous cycle of C57BL/6J mice. *Endocrinology* 1992;130:805-810.
3. **Almeida M, Han L, Martin-Millan M, et al.** Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. *J Biol Chem* 2007;282:27285-27297.
4. **Franceschi C, Capri M, Monti D, et al.** Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev* 2007;128:92-105.
5. **Dimitrijević M, Stanojević S, Vujić V, et al.** Aging oppositely affects TNF-α and IL-10 production by macrophages from different rat strains. *Biogerontology* 2014;15:475-486.
6. **Smallwood HS, López-Ferrer D, Squier TC.** Aging enhances the production of reactive oxygen species and bactericidal activity in peritoneal macrophages by upregulating classical activation pathways. *Biochemistry* 2011;50:9911-9922.
7. **Chen X, Li L, Guo J, et al.** Treadmill running exercise prevents senile osteoporosis and upregulates the Wnt signaling pathway in SAMP6 mice. *Oncotarget* 2016;7:71072-71086.