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Supplemental information

AAV-mediated delivery of osteoblast/osteoclast-regulating miRNAs for osteoporosis therapy

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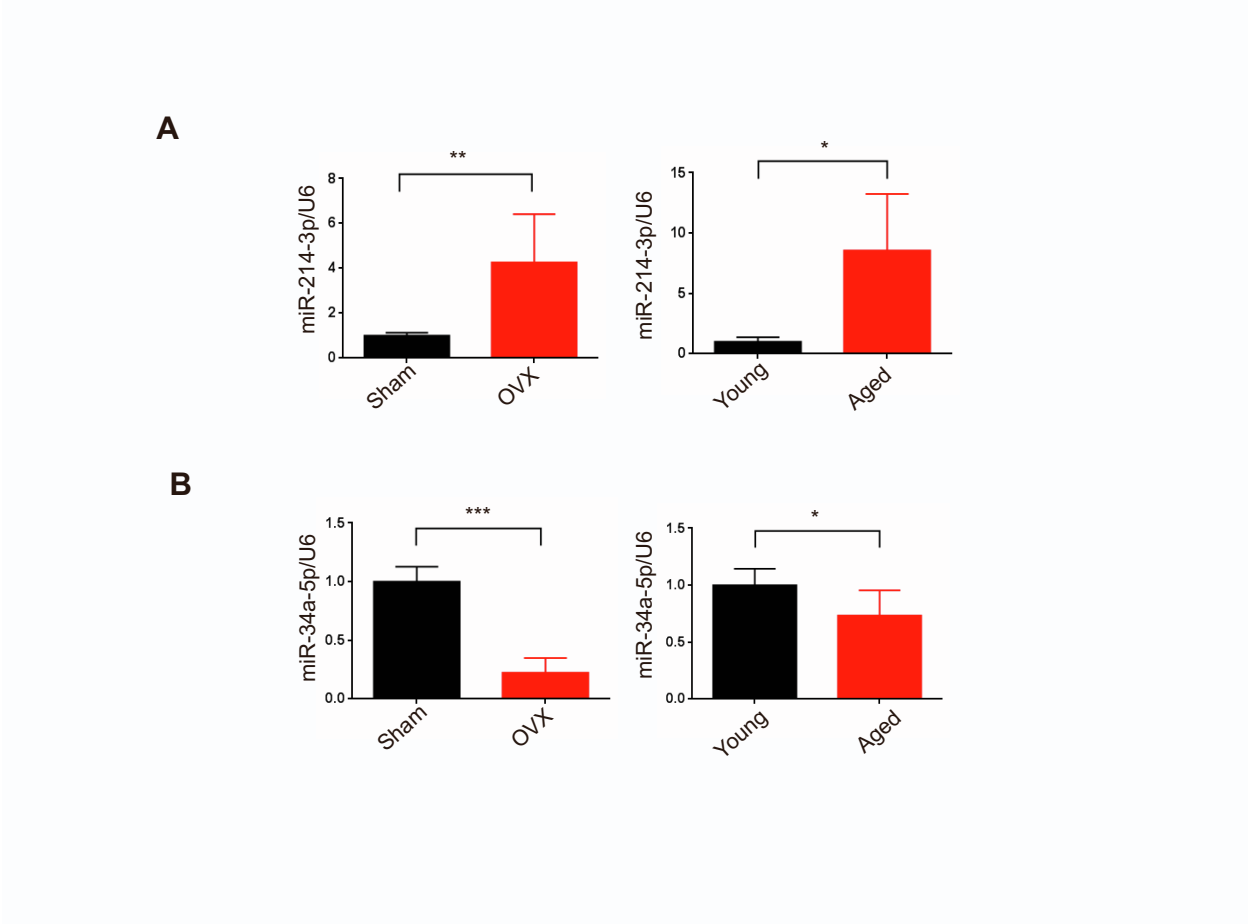
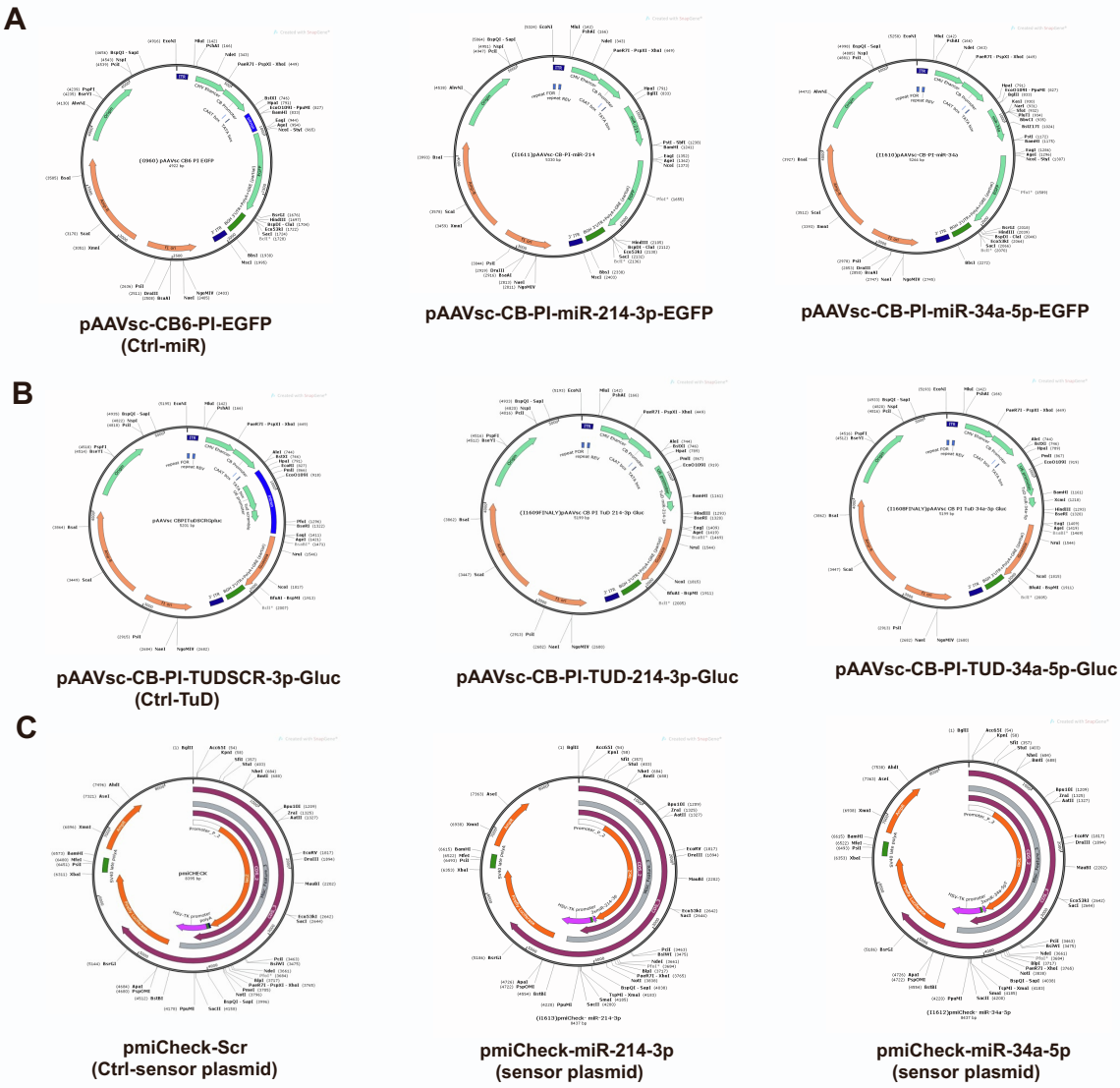


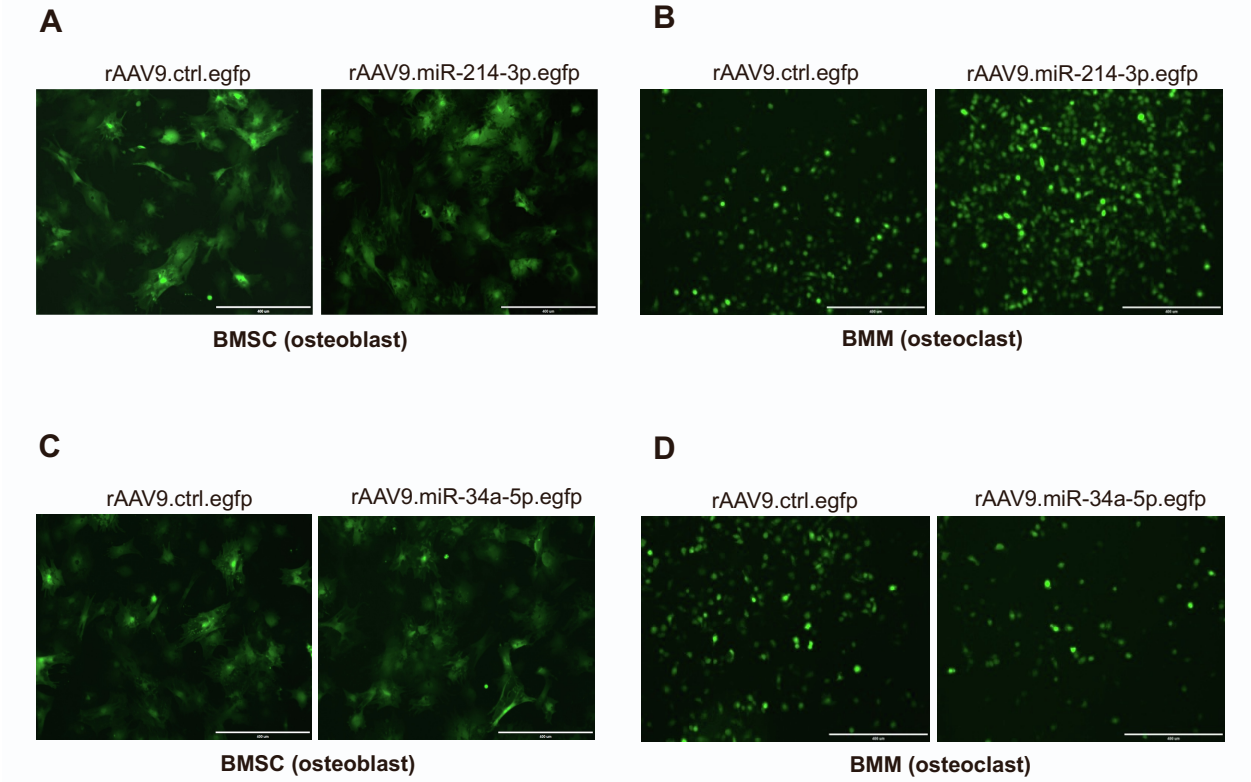
Supplementary Figure 1. **Expression of miR-214-3p and miR-34a-5p in osteoporotic bones.**

Expression of miR-214-3p (A) or miR-34a-5p (B) in the tibia of 16-week-old female mice with Sham or OVX surgery or 2.5-month-old (young) or 24-month-old (aged) male mice was assessed by RT-qPCR. Values represent mean ± SD: *P < 0.05; **P < 0.01; and ***P < 0.001 by an unpaired two-tailed Student's t-test.



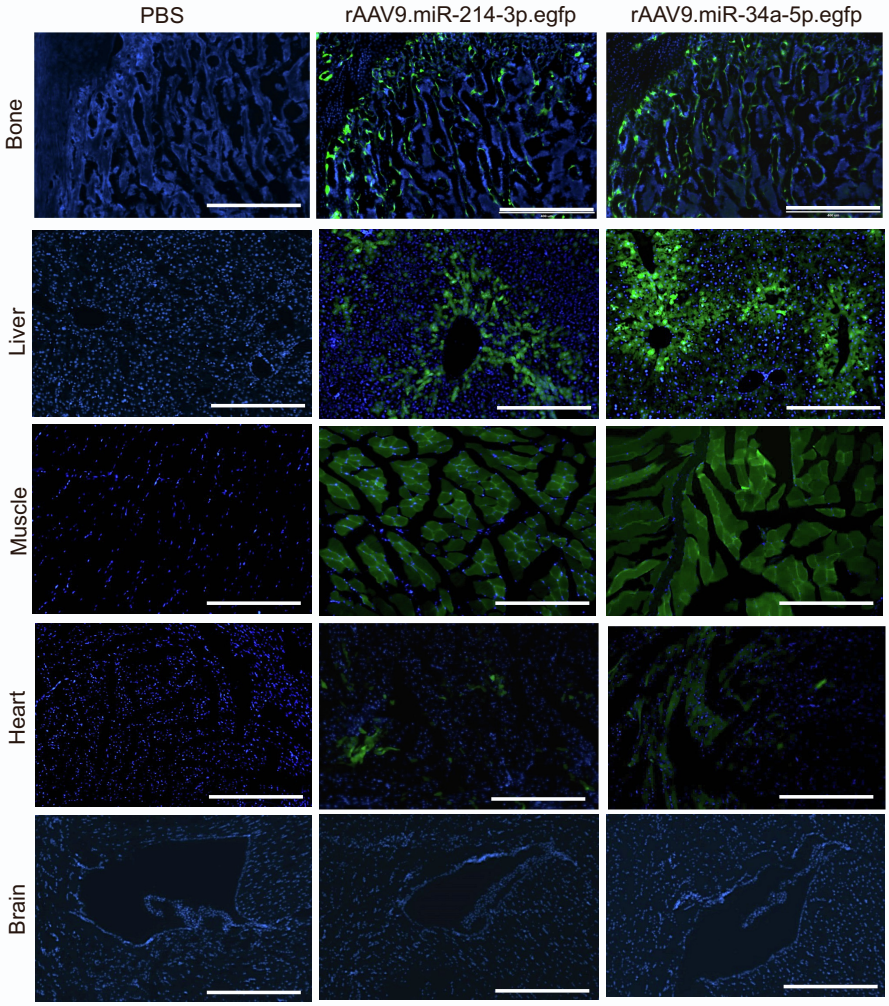
Supplementary Figure 2. Construction of AAV vector genomes and sensor plasmids.

(A) AAV vector genomes containing CBA (CMV enhancer/chicken β-actin) promoter, miRNA (miR-214-3p or miR-34a-5p), and the EGFP gene. (B) AAV vector genomes containing CBA promoter, tough decoy (miR-214-3p TuD or miR-34a-5p TuD), and the *Guassia* luciferase (gLuc) gene. (C) Sensor plasmids containing the complementary sequences of miR-214-3p or miR-34a-5p in the 3'-UTR of Lac Z (β-galactosidase) reporter gene.



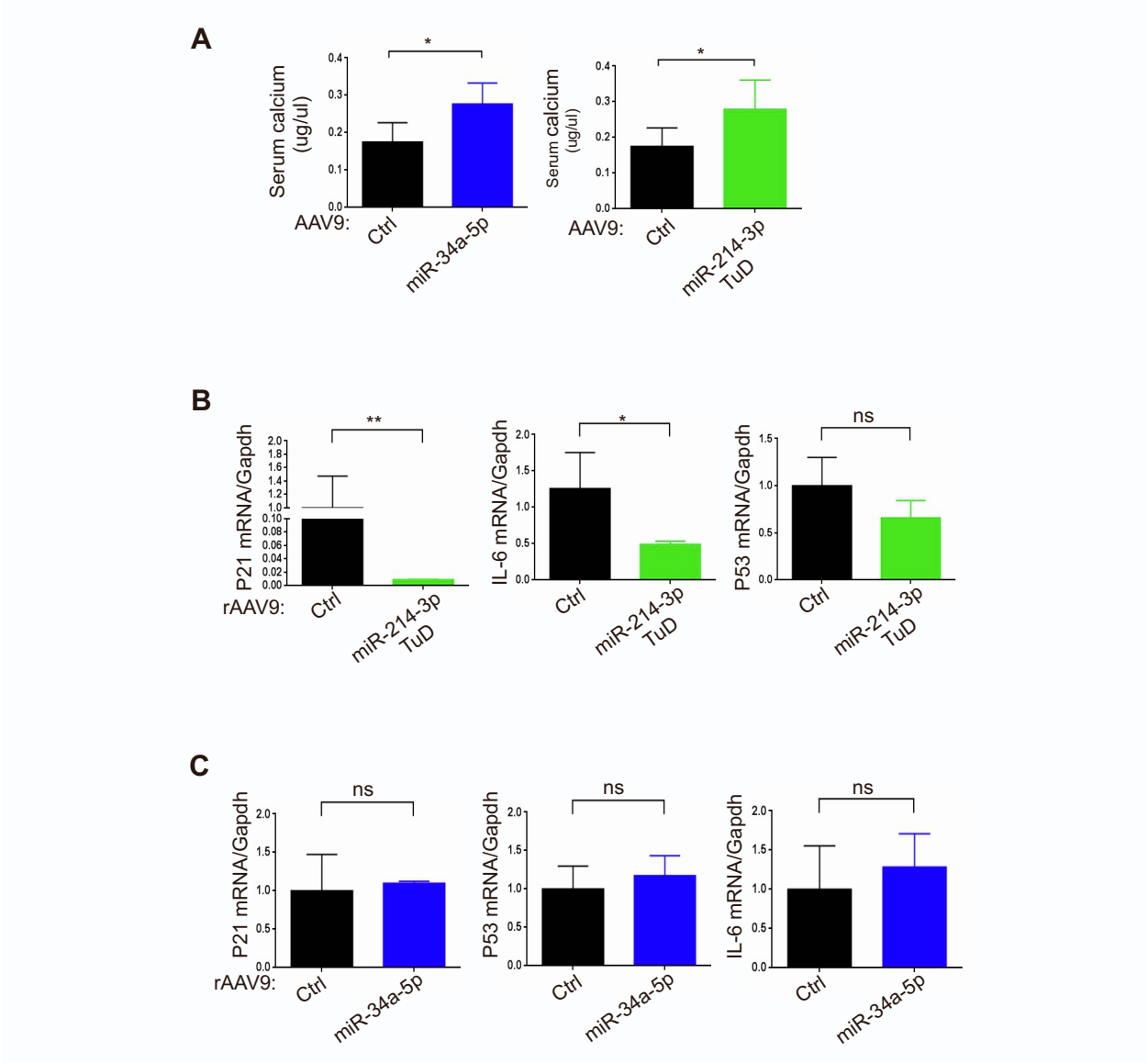
Supplementary Figure 3. *In vitro* transduction efficiency of rAAV9 vectors in the osteoblast and osteoclast.

(A, C) Mouse bone marrow-derived stromal cells (BMSCs) were incubated with rAAV9.egfp carrying ctrl, miR-214-3p or miR-34a-5p for two days and transduction efficiency was assessed by EGFP expression using fluorescence microscopy. (B, D) Two days after treatment with M-CSF and RANKL, mouse bone marrow-derived monocytes (BMMs) were incubated with rAAV9.egfp carrying ctrl, miR-214-3p or miR-34a-5p for two days and transduction efficiency was assessed by EGFP expression using fluorescence microscopy. Scale bars: 400 μm.



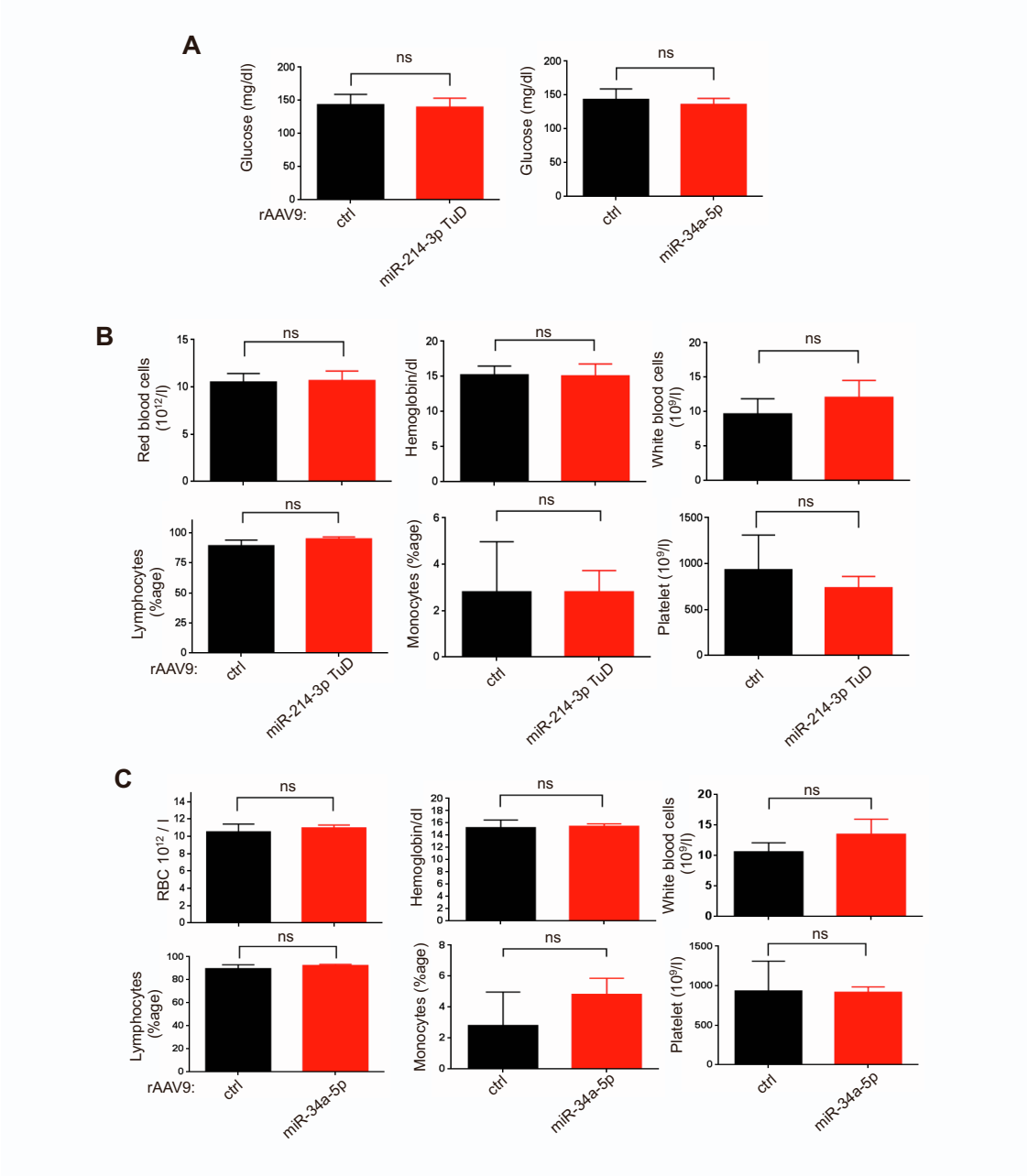
Supplementary 4. Tissue distribution of systemically delivered rAAV9 vectors in mice.

Two-month-old healthy mice were i.v. injected with a single dose of PBS or rAAV9 (5×10^{13} kg/vg) carrying ctrl, miR-214-3p, or miR-34a-5p, and two weeks later, EGFP expression in individual tissues was assessed by fluorescence microscopy. Scale bars: 400 μm.



Supplementary Figure 5. Effects of AAV9 vectors carrying miR-214-3p TuD or miR-34a-5p on serum calcium levels and bone senescence in aged mice.

24-month-old male mice (n = 5) were i.v. injected with a single dose of rAAV9 (5×10^{13} kg/vg) carrying ctrl or miR-214-3p TuD or miR-34a-5p, and eight weeks later, calcium levels in the serum were measured by calorimetric assay (A). mRNA levels of cell senescence marker genes, including p21, p53, and IL-6, were assessed by RT-PCR analysis and normalized to Gapdh (B, C). Values represent mean ± SD: *P < 0.05; **P < 0.01; and ns, not significant by an unpaired two-tailed Student's t-test.



Supplementary Figure 6. Effects of AAV9 vectors carrying miR-214-3p TuD or miR-34a-5p in healthy mice.

Ten-week-old healthy mice were i.v. injected with a single dose of rAAV9 (5×10^{13} kg/vg) carrying ctrl, miR-214-3p TuD, or miR-34a-5p and eight weeks later, tests for blood glucose levels (A) and complete blood count (CBC) (B, C) were performed in AAV-treated mice (n = 5). Values represent mean ± SD: ns, not significant by an unpaired two-tailed Student's t-test.

Supplementary Table 1. Mouse primer sequences for RT-PCR

Name	Forward Primer	Reverse Primer
Runx2	TACAAACCATACCCAGTCCCTGTTT	AGTGCTCTAACCACAGTCCATGCA
Ibsp (Bsp)	CAGGGAGGCAGTGACTCTTC	AGTGTGGAAAGTGTGGCGTT
Sp7 (Osx)	ATGGCGTCCTCTCTGCTTGA	GAAGGGTGGGTAGTCATTTG
Bglap (Ocn)	GCAGCACAGGTCCTAAATAG	GGGCAATAAGGTAGTGAACAG
Pten	AATTCCCAGTCAGAGGCGCTATGT	GATTGCAAGTTCCGCCACTGAACA
Notch1	AACAGTGCCGAATGTGAGTGG	AAGTGACGCAAGAGCACCTAG
Atf4	ATGATGGCTTGGCCAGTG	CCATTTTCTCCAACATCCAATC
Tgif2	CCTCTCGGTGCTGCAGATA	TAGGGTCTTTGCCATCCTTC
Ctsk	AGCAGAACGGAGGCATTGACTC	CCCTCTGCATTTAGCTGCCTTTG
Acp5	GCGACCATTGTTAGCCACATACG	CGTTGATGTCGCACAGAGGGAT
RANK	TGGGTGATTTTCTTTTGGTGGG	CCAAGAACCAGTGCTCGTGA
Gapdh	ACTGAGCAAGAGAGGCCCTA	TATGGGGGTCTGGGATGGAA
Hprt	CTGGTGAAAAGGACCTCTCGAAG	CCAGTTTCACTAATGACACAAACG

Supplementary Table 2. Oligonucleotides and gBlocks used in this study

Oligonucleotides	Sequence(5′to3′)	Aims
TuD34a-5p F	CATCAACACAACCAGCTAAGTCGGACACTGCCACAAG TATTCTGGTCACAGAATACAACACAACCAGCTAAGTCG GACACTGCCACAAG	To generate TuD34a-5p for miR-34a-5p inhibition
TuD34a-5p R	TCATCTTGTGGCAGTGTCCGACTTAGCTGGTTGTGTTG TATTCTGTGACCAGAATACTTGTGGCAGTGTCCGACTT AGCTGGTTGTGTT	
TuDmiR-214-3P F	CATCAACACTGCCTGTCTGGTCGTGCCTGCTGTCAAGT ATTCTGGTCACAGAATACAACACTGCCTGTCTGGTCGT GCCTGCTGTCAAG	To generate TuD214-3p for miR-214-3p inhibition
TuDmiR-214-3P R	TCATCTTGACAGCAGGCACGACCAGACAGGCAGTGTT GTATTCTGTGACCAGAATACTTGACAGCAGGCACGAC CAGACAGGCAGTGTT	
3xmiR-34a-5pT sense	TCGAGACAACCAGCTAAGACACTGCCAACAACCAGCT AAGACACTGCCAACAACCAGCTAAGACACTGCCAGC	To generate miR-34a-5p targeting sites
3xmiR-34a-5pT antisense	GGCCGCTGGCAGTGTCTTAGCTGGTTGTTGGCAGTGT CTTAGCTGGTTGTTGGCAGTGTCTTAGCTGGTTGTC	
3xmiR-214-3p sense	TCGAGACTGCCTGTCTGTGCCTGCTGTACTGCCTGTCT GTGCCTGCTGTACTGCCTGTCTGTGCCTGCTGTGC	To generate miR-214-3p targeting sites
3xmiR-214-3p antisense	GGCCGCACAGCAGGCACAGACAGGCAGTACAGCAGG CACAGACAGGCAGTACAGCAGGCACAGACAGGCAGTC	
miR-34a gBlock	GTCTTTTATTTCAGGTCCCAGATCTTGGCTTCCAAGTG CTGGAGGAGTGTGTCATACCTCGGTAGGGTCCACTAC ACATCTTTCTCCCGCAGCCTCTCCATCTTCCTGTGACT GCGGGCGCCTCAGCCTGGGCTGGCCAGCTGTGAGTA ATTCTTTGGCAGTGTCTTAGCTGGTTGTTGTGAGTATT AGCTAAGGAAGCAATCAGCAAGTATACTGCCCTAGAA GTGCTGCACATTGTTGGGCCGAGAAGGAAAAGGTCAG AGGTCAGCAACGCCCACACCCCTGAGAGGCGCTGGA CTTGCGGAGCTGCTCGACCATACTGGTGGGTATGGGA TGCTGGGGAGGCTGGTGTTACTGCAGGGGATCCGGT GGTGGTGC	To express miR-34a-5p
miR-214 gBlock	GTCTTTTATTTCAGGTCCCAGATCTTTAGTTCCATAATG TTTTAATGTTTAATTCTATTGTGTGTTTCTCTCCTTTCCC TTTATCCCCCTGTCCTTCCCCCCTAAATCACCAAATCT GGAAAACAGGCTGATTGTATCTGTCCCTGAGCAAAGG AAACCTGAAGGACCCAAGGGCCTGGCTGGACAGAGTT GTCATGTGTCTGCCTGTCTACACTTGCTGTGCAGAACA TCCGCTCACCTGTACAGCAGGCACAGACAGGCAGTCA CATGACAACCCAGCCTGAATGACCACCAGCCATTGAA AGAAAGCTGCCCTCACAACATAGCATCTACACCAAGAG CTACAACCACAGTGAGGGGGTTGGGGGGCCTGGGGT TTGAAACTGTTGGCTTATTAAAAGAAAAACTCGTATGTA ATCCCTGCAGGGGATCCGGTGGTGGTGC	To express miR-214-3p