

Supplementary material

Supplementary Figure legends:

Supplementary Figure 1: Gene expression after overexpression, knockdown, or knockout. A, B

The transfection efficiencies of miR-128 mimic or inhibitor. Data are mean \pm SD. $**P < 0.01$ by Student's *t* test. C *miR-128* levels in BMMs from *miR-128^{Oc-/-}* mice. Data are mean \pm SD. $**P < 0.01$ by Student's *t* test. D Representative images of *WT* and *miR-128^{Oc-/-}* male mice at 10 weeks old. E The efficiencies of siRNA of SIRT1. Data are mean \pm SD. $**P < 0.01$ by Student's *t* test.

Supplementary Figure 2: Cell proliferation and apoptosis assay. A BMMs were cultured in the

presence of M-CSF and RANKL with miR-128 mimic or inhibitor. Data are mean \pm SD. Not statistically significant by one-way ANOVA with Tukey's *post hoc* test. B BMMs were isolated from the *WT* and *miR-128^{Oc-/-}* mice. Data are mean \pm SD. Not statistically significant by Student's *t* test. C BMMs derived from *WT* and *miR-128^{Oc-/-}* mice that were transfected with the siCtrl or siSIRT1. Data are mean \pm SD. Not statistically significant by two-way ANOVA.

Supplementary Figure 3: Osteoclastic miR-128 deficiency does not affect osteoblastogenesis. A

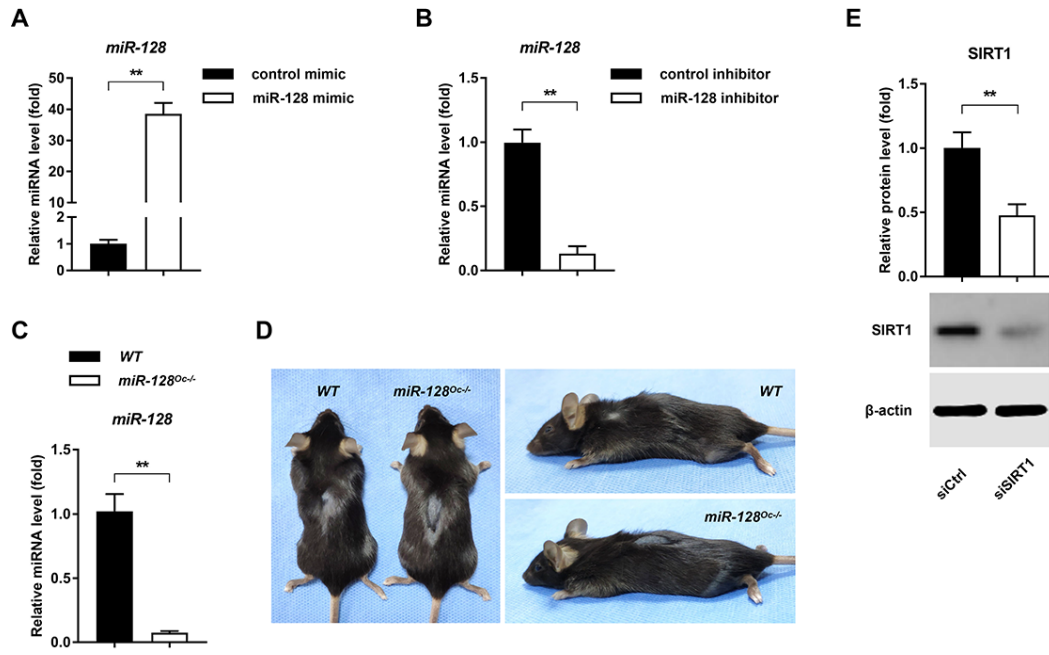
Representative H&E-stained sections (yellow arrows: osteoblasts) of trabecular bone (Tr.B) and histomorphometric analysis of osteoblast surfaces (Ob.S/BS). Scale bars: 100 μ m. Data are mean \pm SEM. Not statistically significant by Student's *t* test. B Mineralizing surface (MS/BS), mineral apposition rate (MAR), bone formation rate (BFR) were analyzed in calcein double-labeled plastic sections of mice vertebrae. Data are mean \pm SEM. Not statistically significant by Student's *t* test. C Serum osteocalcin values tested by ELISA. Data are mean \pm SEM. Not statistically significant by Student's *t* test. D Osteogenic differentiation of osteoblastic stromal cells harvested from *WT* and *miR-128^{Oc-/-}* mice. ALP staining and ARS staining were carried out. E-F Quantitative analyses of the ALP activity and calcium mineralization. Data are mean \pm SD. $**P < 0.01$ by Student's *t* test. G qRT-PCR analysis of mRNA expression of *Runx2*, *Sp7*, *Alp*, and *Ocn*. Data are mean \pm SD. $**P < 0.01$ by Student's *t* test.

Supplementary Figure 4: Quantitative analyses of Western blotting results. A Osteoclastic

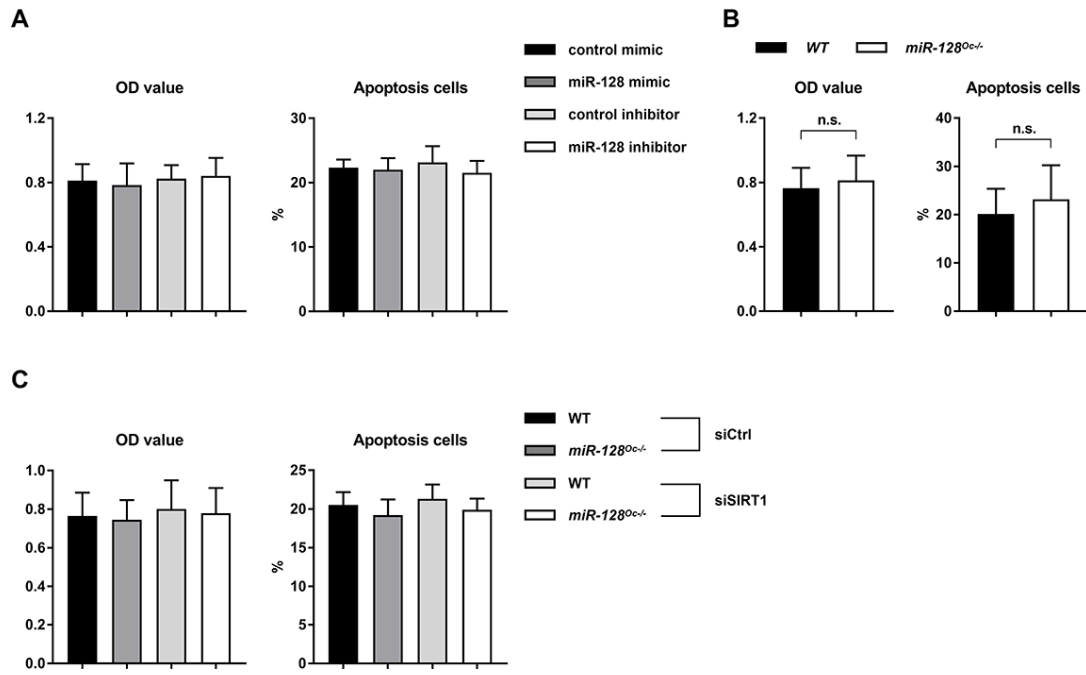
miR-128 deficiency significantly increased SIRT1 protein level, while decreased p65-Acetyl 310 protein level. Data are mean \pm SD. $**P < 0.01$ by Student's *t* test. B SIRT1 knockdown in BMMs significantly recovered the decreased expression of p65-Acetyl 310 induced by osteoclastic miR-128 deficiency. Data are mean \pm SD. $**P < 0.01$ by two-way ANOVA.

Supplementary Figure 5: Osteoclastic miR-128 deficiency does not affect osteoblastogenesis in OVX mice. **A** Representative H&E-stained sections (yellow arrows: osteoblasts) of trabecular bone (Tr.B), and histomorphometric analysis of osteoblast surfaces (Ob.S/BS). Scale bars: 100 μ m. Data are mean \pm SEM. Not statistically significant by two-way ANOVA. **B** Mineralizing surface (MS/BS), mineral apposition rate (MAR), bone formation rate (BFR) were analyzed in calcein double-labeled plastic sections of mice vertebrae. Data are mean \pm SEM. Not statistically significant by two-way ANOVA. **C** Serum osteocalcin values tested by ELISA. Data are mean \pm SEM. Not statistically significant by two-way ANOVA.

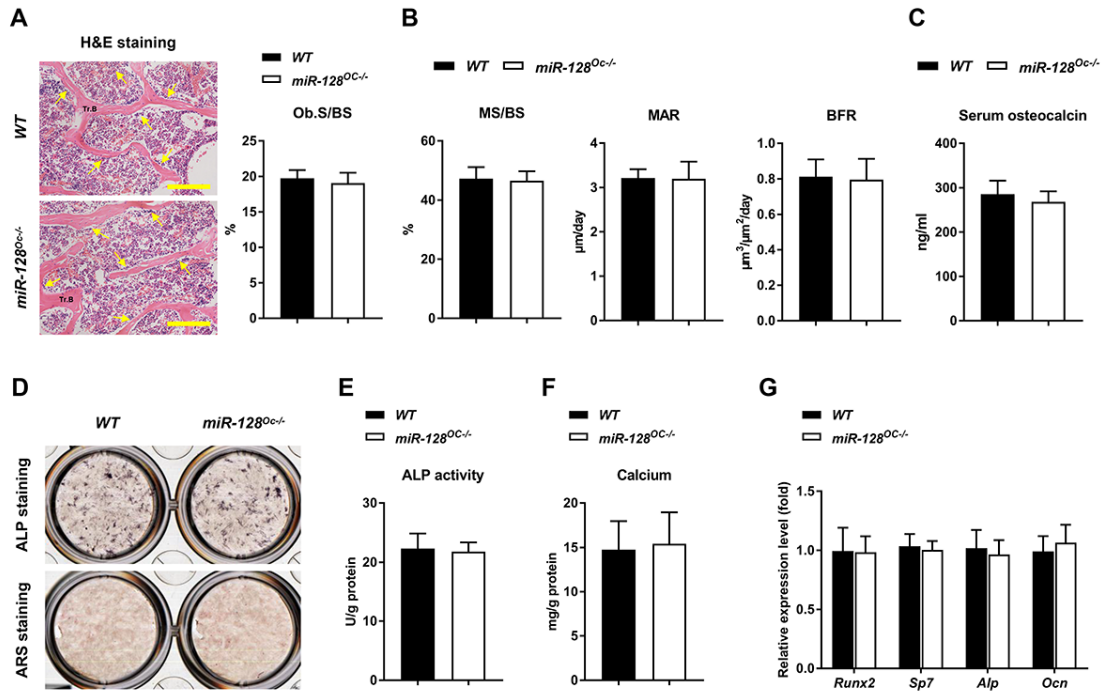
Supplementary Figure 1:



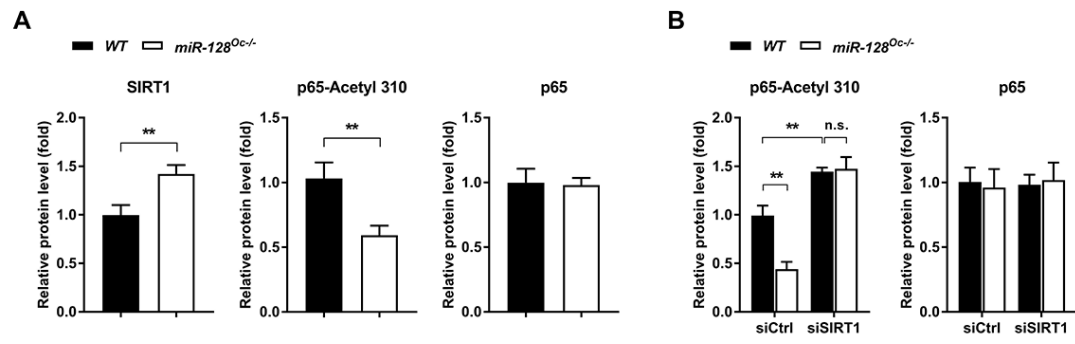
Supplementary Figure 2:



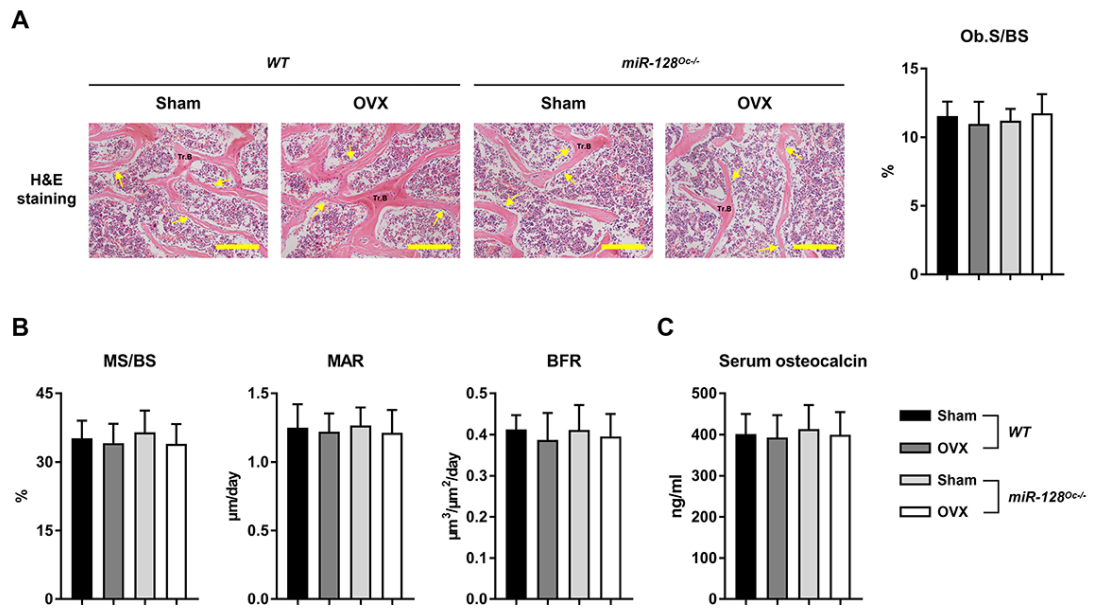
Supplementary Figure 3:



Supplementary Figure 4:



Supplementary Figure 5:



Supplementary Table 1. Sequences of siRNA, probe and primers.

Item	Sequence
siSIRT1	GCTGTTCGTGGAGACATTT
SIRT1 pull down probe	AAGGCGAGCATAGATACCGT
mmu-SIRT1 qRT-PCR primers	Forward: GCTGACGACTTCGACGACG Reverse: TCGGTCAACAGGAGGTTGTCT
mmu-miR-128 qRT-PCR primers	Forward: GGTCACAGTGAACCGGTC Reverse: GTGCAGGGTCCGAGGT
mmu-U6 qRT-PCR primers	Forward: GTGCTCGCTTCGGCAGCACATAT Reverse: AAAATATGGAACGCTTCACGAA
mmu-Nfatc1 qRT-PCR primers	Forward: CCCGTCACATTCTGGTCCAT Reverse: CAAGTAACCGTGTAGCTCCACAA
mmu-Traf6 qRT-PCR primers	Forward: AAAGCGAGAGATTCTTTCCCTG Reverse: ACTGGGGACAATTCCTAGAGC
mmu-Ctsk qRT-PCR primers	Forward: GAAGAAGACTCACCAGAAGCAG Reverse: TCCAGGTTATGGGCAGAGATT
mmu-c-Fos qRT-PCR primers	Forward: CGGGTTTCAACGCCGACTA Reverse: TTGGCACTAGAGACGGACAGA
mmu-c-Src qRT-PCR primers	Forward: GAACCCGAGAGGGACCTTC Reverse: GAGGCAGTAGGCACCTTTTGT
mmu-Tnf- α qRT-PCR primers	Forward: CCCTCACACTCAGATCATCTTCT Reverse: GCTACGACGTGGGCTACAG
mmu-IL-1 qRT-PCR primers	Forward: CGAAGACTACAGTTCTGCCATT Reverse: GACGTTTCAGAGGTTCTCAGAG
mmu-GAPDH qRT-PCR primers	Forward: ATCAAGAAGGTGGTGAAGCA Reverse: AGACAACCTGGTCCTCAGTGT
mmu-Runx2 qRT-PCR primers	Forward: GACCAGTCTTACCCCTCCTA Reverse: GGCAGTGTTCATCATCTGAAA
mmu-Sp7 qRT-PCR primers	Forward: AAAGGAGGCACAAAGAAGC Reverse: CAGGAAATGAGTGAGGGAAG
mmu-Alp qRT-PCR primers	Forward: GCTTTAAACCCAGACACAAG Reverse: AAGAAGAAGCCTTTGAGGTT
mmu-Ocn qRT-PCR primers	Forward: CTCTCTCTGCTCACTCTGCT Reverse: GACTGAGGCTCCAAGGTAG
hsa-miR-128 qRT-PCR primers	Forward: AACTCCAGCTGGGTCACAGTGAACCGGTC

	Reverse: TGGTGTCTGGAGTCG
hsa-U6 qRT-PCR primers	Forward: CTCGCTTCGGCAGCACA
	Reverse: AACGCTTACGAATTTGCGT
hsa-Nfatc1 qRT-PCR primers	Forward: AAAGACGCAGAAACGACG
	Reverse: TCTACTAACGGGACATCAC
hsa-GAPDH qRT-PCR primers	Forward: ATCAAGAAGGTGGTGAAGCA
	Reverse: GTCGCTGTTGAAGTCAGAGGA
