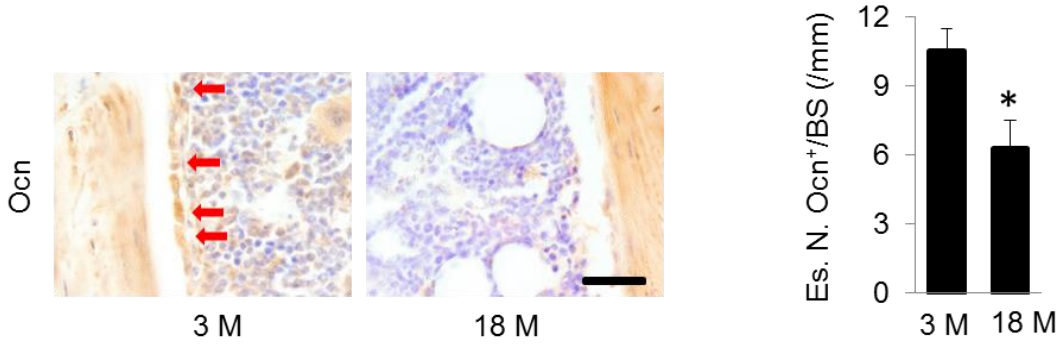
SUPPLEMENTAL MATERIAL

Supplemental Figures

Supplemental Figure 1:



Supplemental Figure 1: Representative images of osteocalcin immunohistochemical staining and quantification of osteoblast number on endosteal bone surface of distal femora of 3 months old (3 M) and 18 months old (18 M) female C57BL/6 mice. Scale bar: 50 µM. Es.N.Ocn$^+$/BS, number of osteocalcin$^+$ cells per endosteal bone surface. n=5 per group; *p < 0.05. (Student's *t*-test).

Supplemental Figure 2:

A.

CCCTCTTGGTCCGCATGTGTGTGTTCCTCCTGCCTTACCCACCATCAGTTTCATGTAGCTGGTTT

GTTGTTTCTGCGTGATCCCTGGGCTGCTTTCTGTTGCTTTTTCCATAGAAAGTACCTACCTGTTG

TGCCAAGAGAGTGAACCCTTCCCTGCTCCCTCTCTCACATCCCTTGCATGGTGGAGGGTGAGCTC

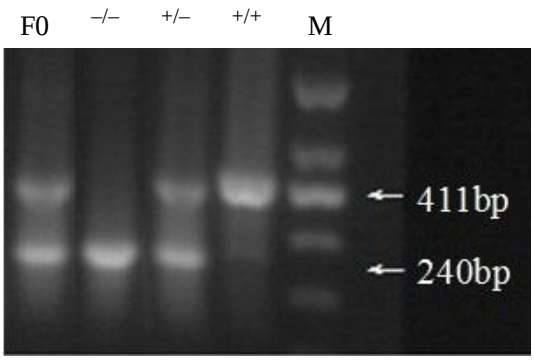
TCTGAAAACCCCTCCCACATGCAGGGTTTGCAGGATGGTGAGCTTCAGCTTTCCTTGCTCTCTTG

TGCATGGTGGACATATGCATACACAACTTCATAAAGAAAACCCCACATACCCAAGGAAACAGCCA

TCTGTCCTGTCTTCCACCTGACATGCACATCTCACAGTTTTCTTTCTCCAGGGAGGGCTCATGTG

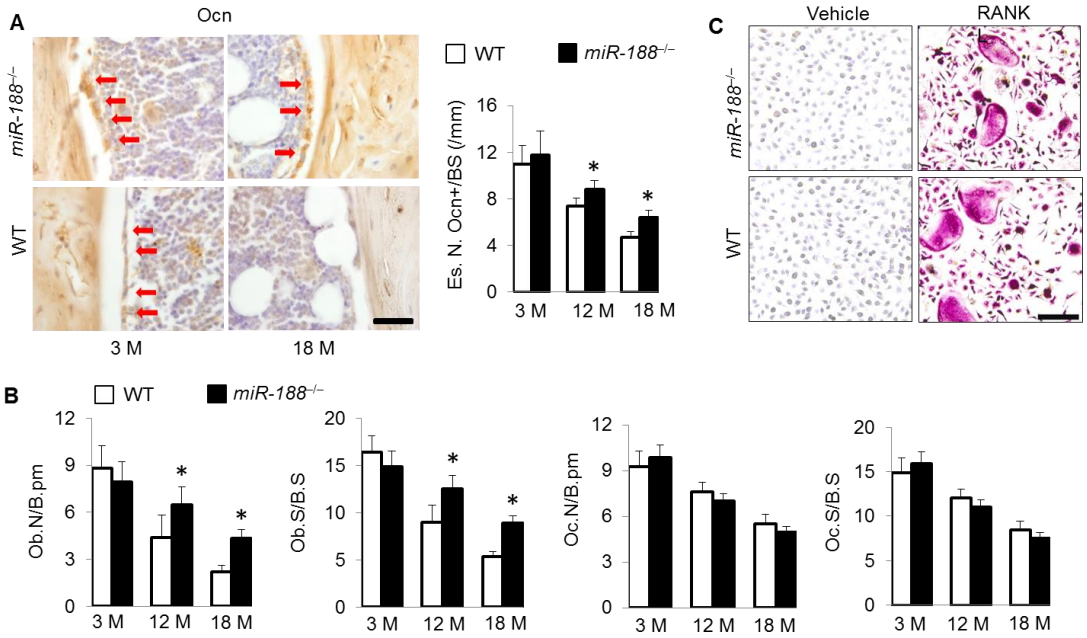
TCCCATGCTGCCTTTGAACTCCCTGCATAGC -171bp

B.



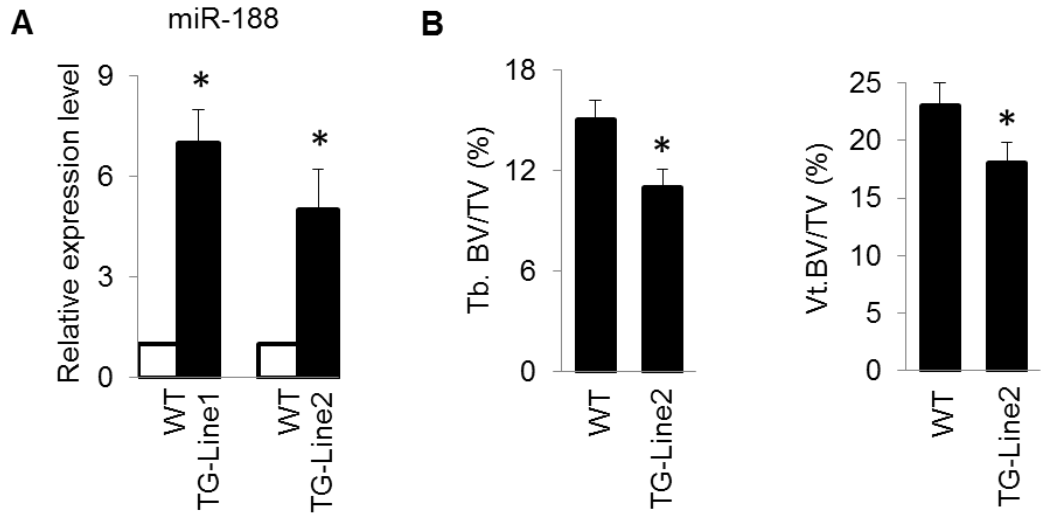
Supplemental Figure 2: The miR-188 knock out mice construction. (A) The sequence in green showed the target bonding sites of miR-188 TALEN plasmids Left-pCS2-PEAS and Right-pCS2-PERR; The Sequences in red indicate mature miR-188; The sequence in yellow showed the Primer sites for PCR; The sequences underlined indicate the knockout sequences. **(B)** **(B)** Female founder mouse (F0) harboring TALEN-induced mutations was crossed to male wild-type mouse to generate F1 offspring. F1 offspring were used to mate to generate: homozygote$^{-/-}$, heterozygote$^{+/-}$, wild-type $^{+/+}$; (M, 1000-bp marker DNA.)

Supplemental Figure 3



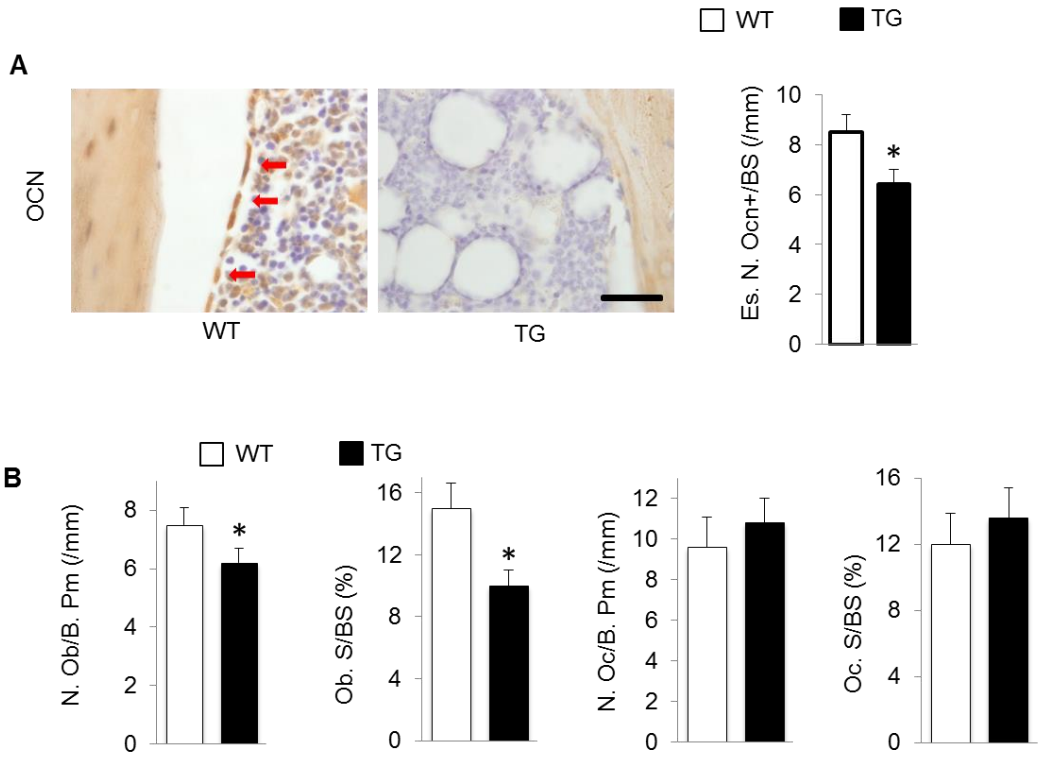
Supplemental Figure 3: Aged miR-188$^{-/-}$ mice shows increased osteoblasts on bone surface without affecting osteoclast. (A) Representative images of osteocalcin immunohistochemical staining of femora from 3 months (3 M) and 18 months (18 M) old wild-type (WT) and miR-188 knockout (miR-188$^{-/-}$) mice. Scale bar: 50μM. Quantification data of osteocalcin^{+} cells in endosteal bone surface. Es.N.Ocn^{+}/BS, number of osteocalcin^{+} cells per endosteal bone surface. n=5 per group; **(B)** Bone histomorphometric analysis of femora. Number of osteoblasts per bone perimeter (N.Ob/B.Pm), osteoblast surface per bone surface (Ob.S/BS), number of osteoclasts per bone perimeter (N.Oc/B.Pm), and osteoclast surface per bone surface (Oc.S/BS), were measured. n=5 per group; **(C)** Osteoclast differentiation of monocytes cultured with M-CSF and RANKL from WT and miR-188$^{-/-}$ mice. *p < 0.05. (Student's *t*-test)

Supplemental Figure 4



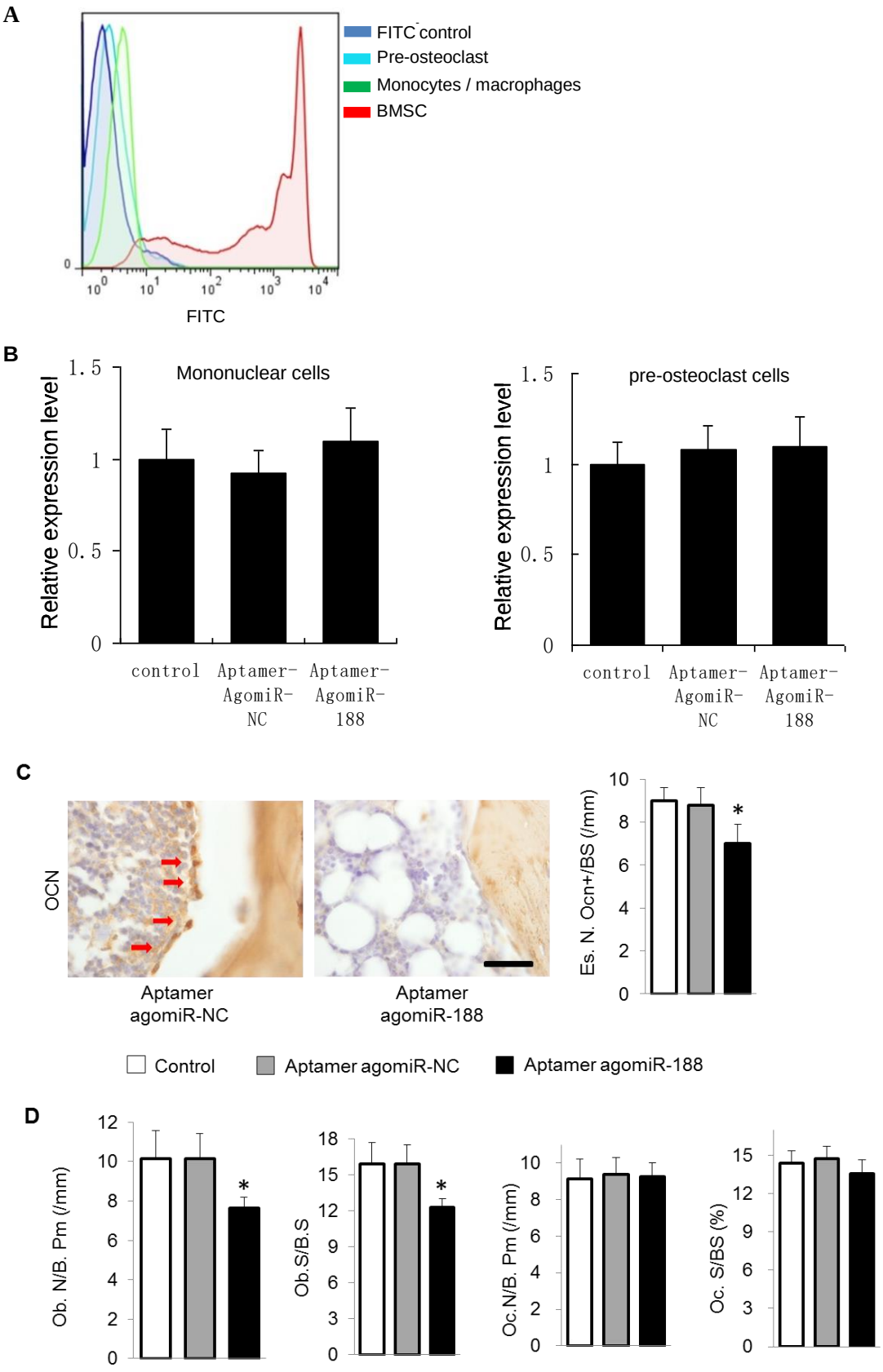
Supplemental Figure 4: miR-188 expression and bone volume in miR-188 transgenic (TG) mice and wild-type (WT) mice. (A) Expression of miR-188 in osterix^{+} osteoprogenitor cells from 6 month-old WT, TG line1 and TG line2 female mice was determined using real-time RT-PCR. **(B)** Quantitative micro-CT analysis of femora and vertebra from TG line 2 mice. Ratio of trabecular bone volume to tissue volume (Tb.BV/TV); Ratio of L4 vertebra bone volume to tissue volume (Vt.BV/TV) are shown**.** n=6 per group; *$p < 0.05$. (Student's *t*-test)

Supplemental Figure 5



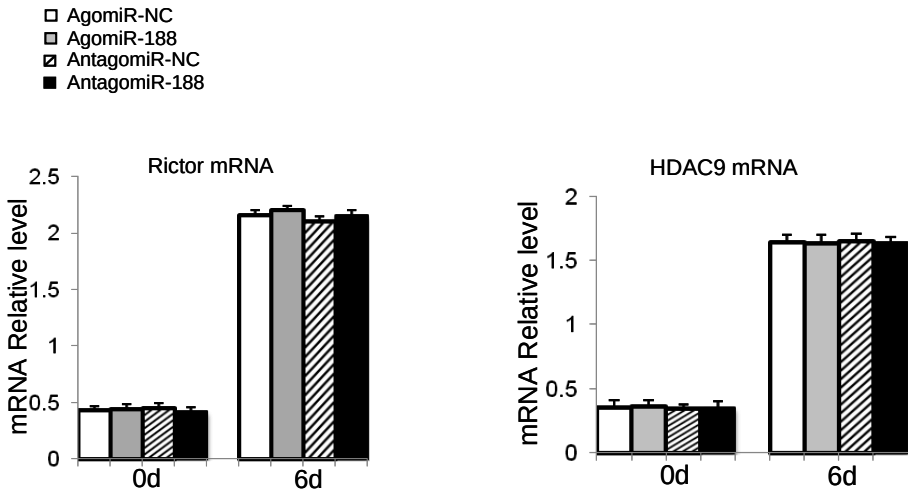
Supplemental Figure 5: miR-188 transgenic (TG) mice shown decreased osteoblasts number and surface in bone surface without affecting osteoclasts (A) Representative images of osteocalcin immunohistochemical staining of femora from 6 months old WT and TG female mice. Scale bar: 50μM. Quantification data of osteocalcin$^+$ cells in endosteal bone surface. Es.N.Ocn$^+$/BS, number of osteocalcin$^+$ cells per endosteal bone surface. n=5 per group. **(B)** Bone histomorphometric analysis of femora. Number of osteoblasts per bone perimeter (N.Ob/B.Pm), osteoblast surface per bone surface (Ob.S/BS), number of osteoclasts per bone perimeter (N.Oc/B.Pm), and osteoclast surface per bone surface (Oc.S/BS), were measured. n=6 per group; *$p < 0.05$. (Student's *t*-test).

Supplemental Figure 6:



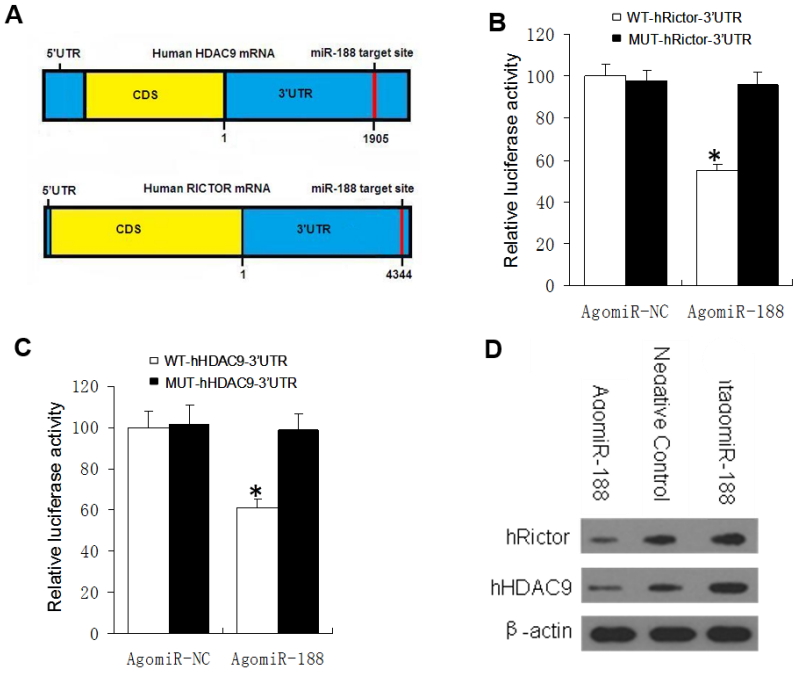
Supplemental Figure 6: Mice with BMSCs-specific overexpression of miR-188 using aptamer delivery system presented reduced bone formation and unchanged bone resorption. **(A)** The red curve represents the BMSCs incubated with fluorescein isothiocyanate (FITC) aptamer; the cyan curve and green curve represent the pre-osteoclasts and mononuclear cells incubated with FITC aptamer respectively, the blue curve represents the FITC negative control. **(B)** Expression of miR-188 in mononuclear and pre-osteoclast cells of mice was determined using real-time RT-PCR. NC: negative control. **(C)** Representative images of osteocalcin immunohistochemical staining of femora. Scale bar: 50 μM. Quantification data of osteocalcin$^+$ cells in endosteal bone surface. Es.N.Ocn$^+$/BS, number of osteocalcin$^+$ cells per endosteal bone surface. n=8 per group. **(D)** Bone histomorphometric analysis of femora. Number of osteoblasts per bone perimeter (N.Ob/B.Pm), osteoblast surface per bone surface (Ob.S/BS), number of osteoclasts per bone perimeter (N.Oc/B.Pm), and osteoclast surface per bone surface (Oc.S/BS), were measured. n=8 per group; *$p < 0.05$. (ANOVA).

Supplemental Figure 7:



Supplemental Figure 7: MiR-188 regulates HDAC9 and Rictor via post-transcription mechanism. The levels of HDAC9 and Rictor mRNA in BMSCs cells transfected with agomiR-188.were determined using qRT-PCR and normalized to β-actin. n=5 per group.

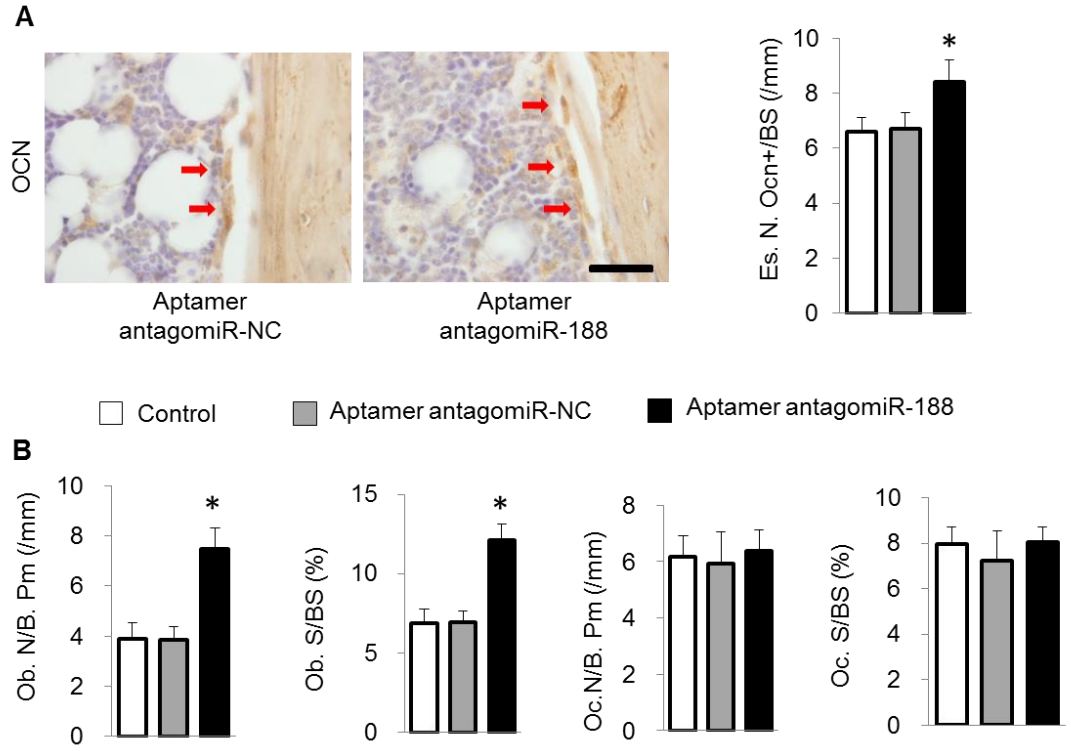
Supplemental Figure 8:



Supplemental Figure 8: miR-188 targets HDAC9 and Rictor in human BMSCs

(A) The predicted traget sites of miR-188 in human HDAC9 and Rictor mRNA. (B and C) Luciferase reporter gene assay-based evaluation of the miR-188 targeting sites in HDAC9 and Rictor mRNA in BMSCs. NC, negative control. n = 5 per group; *P < 0.05. (Student's *t*-test). (D) Western blot for HDAC9 and Rictor protein in human BMSCs after transfection of agomiR-188 and antagomiR-188. Data are representative of 3 independent experiments.

Supplemental Figure 9:



Supplemental Figure 9: Injection of Aptamer-antagomiR-188 into bone marrow stimulated bone formation in aged mice without affecting bone resorption. (A) Representative images of osteocalcin immunohistochemical staining of femora from antagomiR-188 aptamer treated mice. Scale bar: 50 μM. Quantification data of osteocalcin$^+$ cells in endosteal bone surface. Es.N.Ocn$^+$/BS, number of osteocalcin$^+$ cells per endosteal bone surface. n=5 per group. **(D)** Bone histomorphometric analysis of femora from aptamer treated mice. Number of osteoblasts per bone perimeter (N.Ob/B.Pm), osteoblast surface per bone surface (Ob.S/BS), number of osteoclasts per bone perimeter (N.Oc/B.Pm), and osteoclast surface per bone surface (Oc.S/BS), were measured. n=6 per group; *p < 0.05. (ANOVA).

Supplemental Table 1. The alignment of miR-188 with WT and MUT 3'UTR region showing in complementary pairing.

mHDAC9 WT	5'-AAUCUCAUAAAAAAGGGAUA-3'
miR-188	3'-GGGAGGUGGUACGUUCCCUAC-5'
mHDAC9 Mut	5'-AAUCUCAUAAAAAATGCGAUA-3'
mRictor WT-1	5'-UUUGAAAAGUGGUAAGGGAUU-3'
miR-188	3'-GGGAGGUGGUACGUUCCCUAC-5'
mRictor Mut-1	5'-UUUGAAAAGUGGUAACGTAUU-3'
mRictor WT-2	5'-CAUAAUCUUAUCAAAGGGAUU-3'
miR-188	3'-GGGAGGUGGUACGUUCCCUAC-5'
mRictor Mut-2	5'-CAUAAUCUUAUCAAACGTAUU-3'
hRictor WT	5'-CATAATCTTATCAAAGGGATA-3'
miR-188	3'-GGGAGGUGGUACGTTCCCTAC-5'
hRictor Mut	5'-CATAATCTTATCAAACGCATA-3'
hHDAC9 WT	5'-CAATCTCATAAAAAAGGGATA-3'
miR-188	3'-GGGAGGUGGUACGUUCCCUAC-5'
hHDAC9 Mut	5'-CAATCTCATAAAAAACGCATA-3'

The complementary nucleotides are labeled in green. The mutated nucleotides are labeled in red.

Supplemental Table 2. Nucleotide sequences of primers for WT and mutant reporter plasmids.

Gene	Acc. No	Primer sequence(5'to3')
mRictor UTR1	NM_030168	F: GCTCTAGATGTTCCATTACTAGCCTGTC
		R: GGCCGGCCAACACCAGAACCTCCAAA
Mutant mRictor UTR1		F: TTGAAAAGTGGTAACGTATTATAAAGAGGAT
		R: ATCCTCTTTATAATACGTTACCACTTTTCAA
Rictor mUTR2	NM_030168	F: GCTCTAGAAATGGTTTTGCTCACTTT
		R: GGCCGGCCAGTACATTTTATTAACAATG
Mutant mRictor UTR2		F: ATAATCTTATCAAACGTATTCATTGTTAAT
		R: ATTAACAATGAATACGTTTGATAAGATTAT
mHDAC9	NM_ 001271386	F: CATCTAGAAGGCAATTTTCCCTATCA
		R: GGCCGGCCTGTTCCTCCCCAAATAAA
Mutant mHDAC9		F: ATCTCATAAAAAAATGCATAGTGCATCTTT
		R: AAAGATGCACTATGCATTTTTTTATGAGAT
hHDAC9	NM_ 001204144	F: CATCTAGAGTTAGTATATTCCTTCAT
		R: GGCCGGCCCTCTCTCAAATGACATTA
Mutant hHDAC9		F: ATCTCATAAAAAACGCATAGTGCATCTTTT
		R: AAAAGATGCACTATGCGTTTTTTATGAGAT
hRictor	NM_ 152756	F: GCTCTAGACATTGAGTTGTGTATAAT
		R: GGCCGGCCTAAGAATTTTAAGTACAT
Mutant hRictor		F: ATAATCTTATCAAACGCATACATTGTTAAT
		R: ATTAACAATGTATGCGTTTGATAAGATTAT

Note: F, forward primer; R, reverse primer; Acc. No, Genbank accession numbers;

Supplemental Table 3. Nucleotide sequences of primers used for quantitative RT-PCR detection for microRNA

	Primer	Primer sequence(5' to 3')
miR-188a	RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGC ACTGGATACGAC CCCTCC
	Forward	GCCGCCATCCCTTGCATG
	Reverse	CCAGTGCAGGGTCCGAGGTA
U6	RT primer	GAACGCTTCACGAATTTGCGTGTCAT
	Forward	CTCGCTTCGGCAGCACA
	Reverse	AACGCTTCACGAATTTGCGT

Supplemental Table 4. Nucleotide sequences of primers used for quantitative RT-PCR detection for mRNA

Gene	Acc. No	Primer sequence(5'to3')	Size
PPARγ (mouse)	NM_ 001127330	F: GACCACTCGCATTCCTTT	18
		R: CCACAGACTCGGCACTCA	18
Fabp4 (mouse)	NM_ 024406	F: AAATCACCGCAGACGACA	18
		R: CACATTCCACCACCAGCT	18
Runx2 (mouse)	NM_ 001146038	F: ACTTCCTGTGCTCCGTGCTG	20
		R: TCGTTGAACCTGGCTACTTGG	21
β-actin (mouse)	NM_007393	F: CTGTCCCTGTATGCCTCTG	19
		R: TGATGTCACGCACGATTT	18
HDAC9 (mouse)	NM_ 024124	F: GATGATGATGCCTGTGGT	18
		R: AGTTCCTTGATGTGCTCC	18
Rictor (mouse)	NM_030168	F: CGGCGAATCAGAACACTT	18
		R: TGAGCCTTCCACAACCAA	18
Osterix (mouse)	NM_130458	F: ACCAGGTCCAGGCAACAC	18
		R: GCAAAGTCAGATGGGTAAGTAG	22

Note: F, forward primer; R, reverse primer; Acc. No, Genbank accession numbers; Size, primer size.