Table S1 Patients information

Patient ID	Age	Height(m)	Weight(kg)	BMI *	T-score	Serum ALP(U/L)**	Frequence of Exercise/week
1	58	155	65	27.05515088	0.61	101	1
2	62	165	70	25.71166208	-0.07	81	2
3	65	156	74	30.40762656	-0.04	90	3
4	60	150	65	28.88888889	0.13	80	3
5	58	154	66	27.82931354	0.37	111	1
6	58	162	62	23.62444749	0.87	178	3
7	65	163	63	23.71184463	1.62	182	3
8	63	155	70	29.13631634	-0.45	199	2
9	58	157	61	24.74745426	-0.75	190	3
10	59	158	62	24.8357635	-0.83	192	1
11	63	155	61	25.39021852	1.35	81	1
12	58	152	65	28.13365651	1.04	61	1
13	59	161	62	23.91883029	0	72	2
14	65	162	63	24.00548697	-0.14	98	2
15	60	155	66	27.47138398	0.18	43	2
16	62	155	60	24.97398543	-3.02	299	3
17	58	153	61	26.05835362	-2.71	165	1
18	61	158	60	24.03460984	-3.24	151	3
19	65	162	61	23.24340802	-3.37	192	1
20	62	161	62	23.91883029	-2.90	201	1
21	62	164	72	26.76977989	-2.88	232	0
22	59	163	71	26.72287252	-2.66	245	1
23	60	161	66	25.46198063	-2.51	281	0
24	61	155	52	21.64412071	-3.05	291	1
25	65	157	66	26.77593411	-2.66	271	1
26	58	158	61	24.43518667	-2.75	176	1
27	65	162	62	23.62444749	-2.83	165	0
28	63	163	68	25.59373706	-3.03	254	2
29	59	151	61	26.75321258	-3.24	171	1
30	59	155	62	25.80645161	-2.95	251	1

*: BMI formula: BMI = (Weight in Kilograms / (Height in Meters x Height in Meters))
**: reference value: 30 – 150 U/L

Figure S1

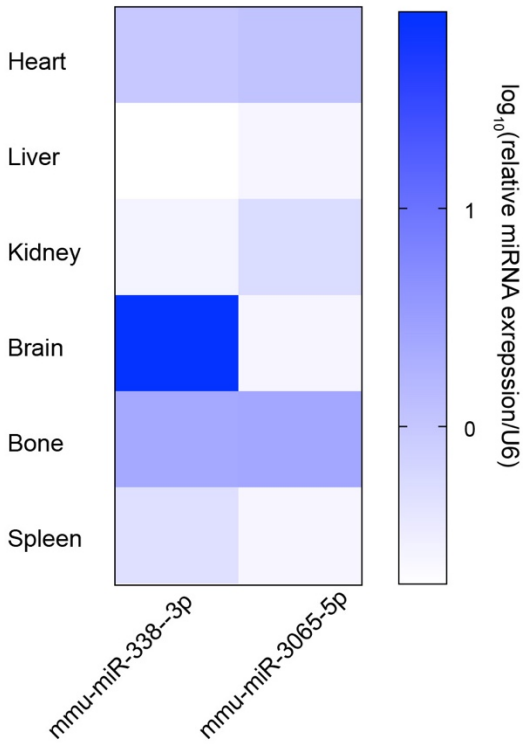


Figure S1 Enrichment of miR-338-3p and miR-3065-5p in different tissues. Expression of each miRNA was normalized to its expression in heart.

Figure S2

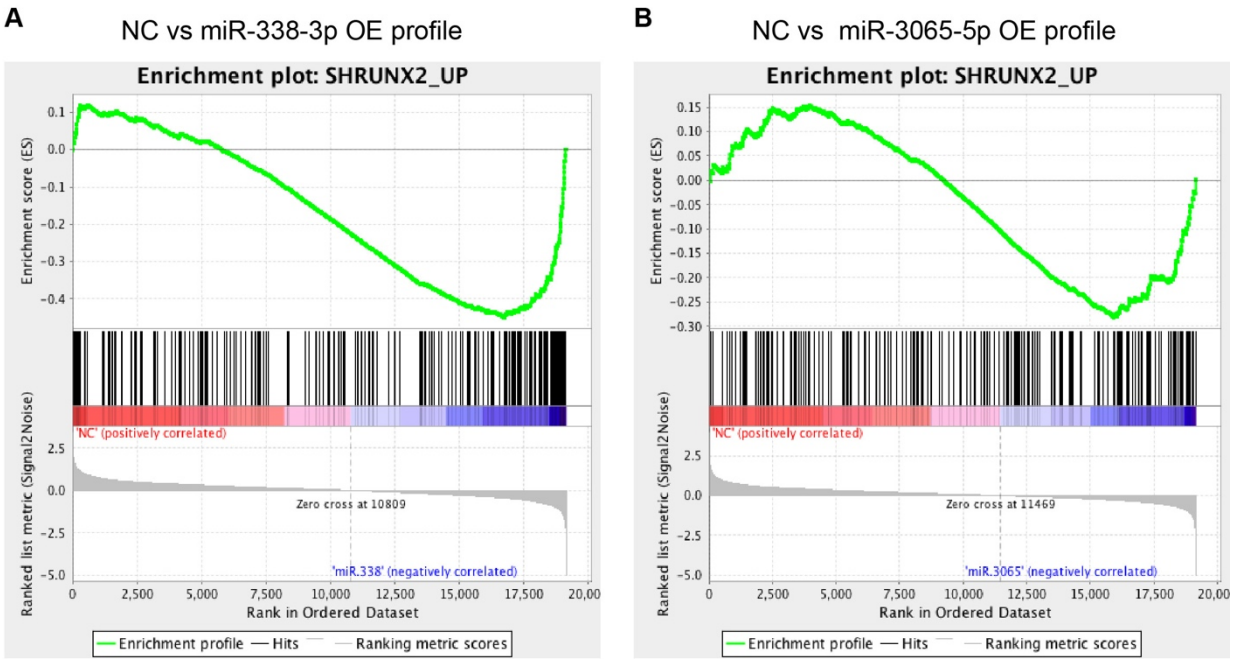


Figure S2 GSEA assays comparing miR-338-3p (A) or miR-3065-5p (B) overexpression profile with Runx2 knockdown (shRunx2) in MC3T3-E1 profile.

Figure S3

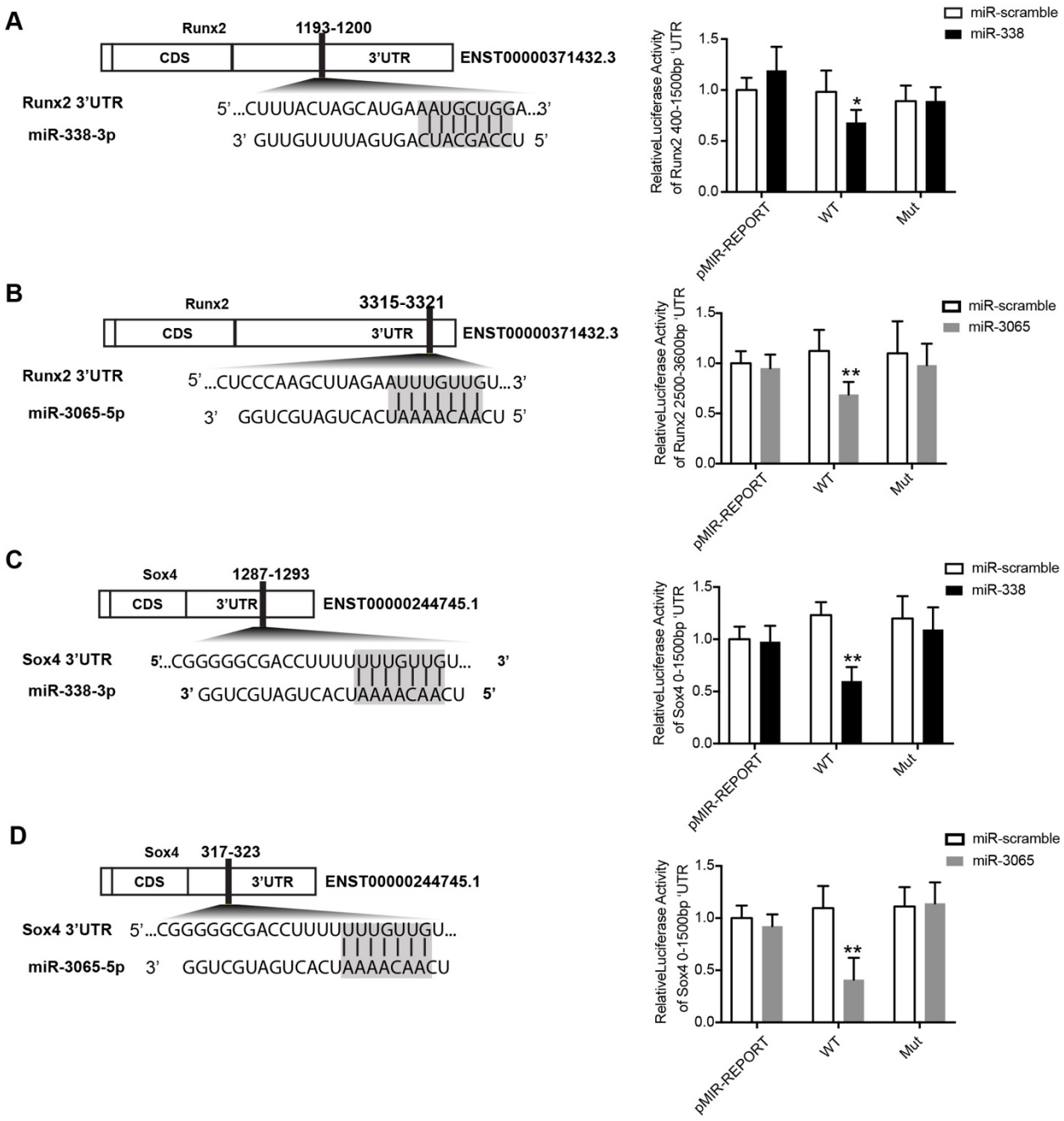


Figure S3 Dual luciferase assay confirming miR-338-3p and miR-3065-5p directly bind to the 3'UTRs of Runx2 and Sox4. (n=3 per group, t-test , *p<0.05, ** p<0.01)

Figure S4

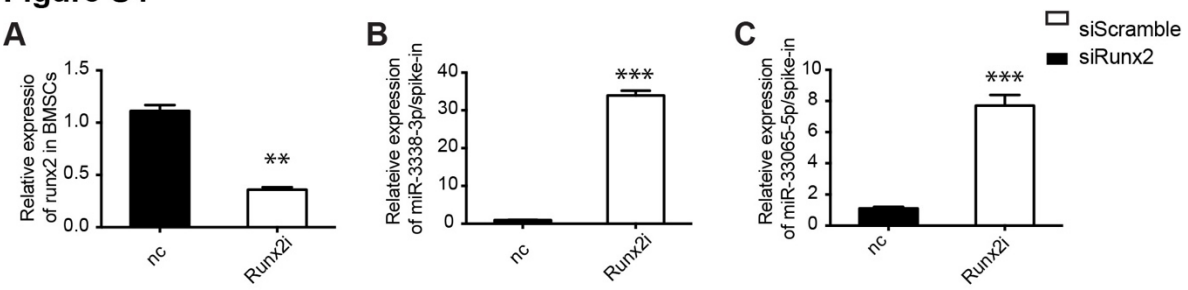


Figure S4 Knockdown of Runx2 in BMSCs up-regulate the enrichment of miR-338-3p (B) and miR-3065-5p (C) in the medium. (n=3 per group, t-test , *p<0.05, ** p<0.01)

Figure S5

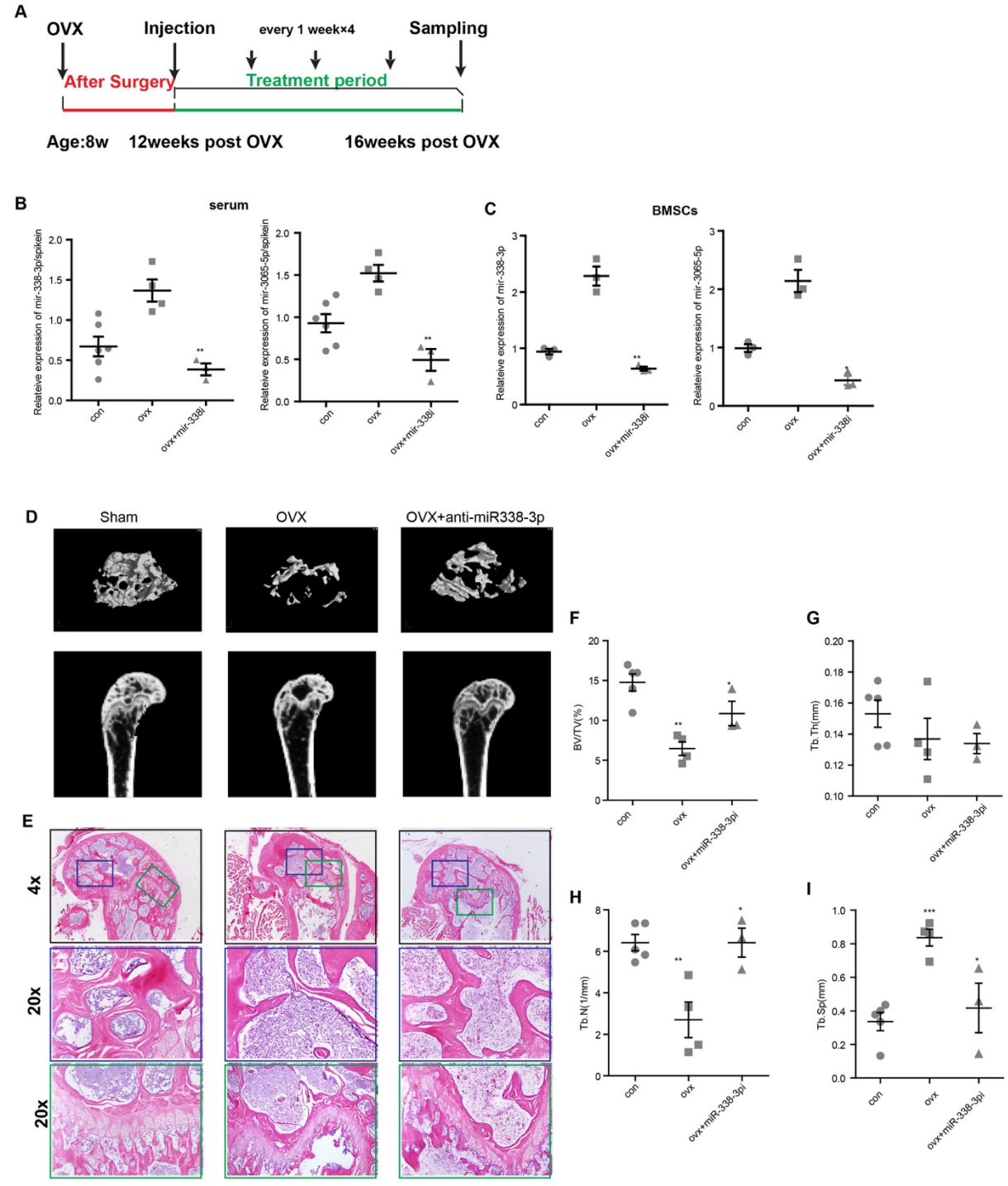

A
OVX
Injection
every 1 week×4
Sampling
After Surgery
Treatment period
Age:8w
12weeks post OVX
16weeks post OVX
B
serum
Relateive expression of mir-338-3p/spikein
Relateive expression of mir-3065-5p/spikein
con
ovx
ovx+mir-338i
C
BMSCs
Relateive expression of mir-338-3p
Relateive expression of mir-3065-5p
D
Sham
OVX
OVX+anti-miR338-3p
E
4x
20x
20x
F
BV/TV(%)
G
Tb.Th(mm)
H
Tb.N(1/mm)
I
Tb.Sp(mm)
ovx+miR-338-3pi

Figure S5 Inhibition of the miR-338-3p in serum attenuated osteoporosis progression *in vivo*. (A) A schematic diagram illustrating the experimental design for the timeline of intravenous injection of miR-338-3p inhibitor (miR-338-3pi). Expression level of miR-338-3p and miR-3065-5p in serum **(B)** and BMSCs **(C)** isolated from different groups of mice on 16 weeks post OVX. Representative micro-CT **(D)** and HE staining **(E)** images in sham, OVX and OVX mice injected with miR-338-3p inhibitor. Bone volume fraction (BV/TV) **(F)**, Trabecular Thickness (Tb.Th) **(G)**, average trabecular number (Tb.N) **(H)** and trabecular spacing (Tb.Sp) **(I)** in femurs isolated from different groups. (n=3 per group, t-test)

Figure S6

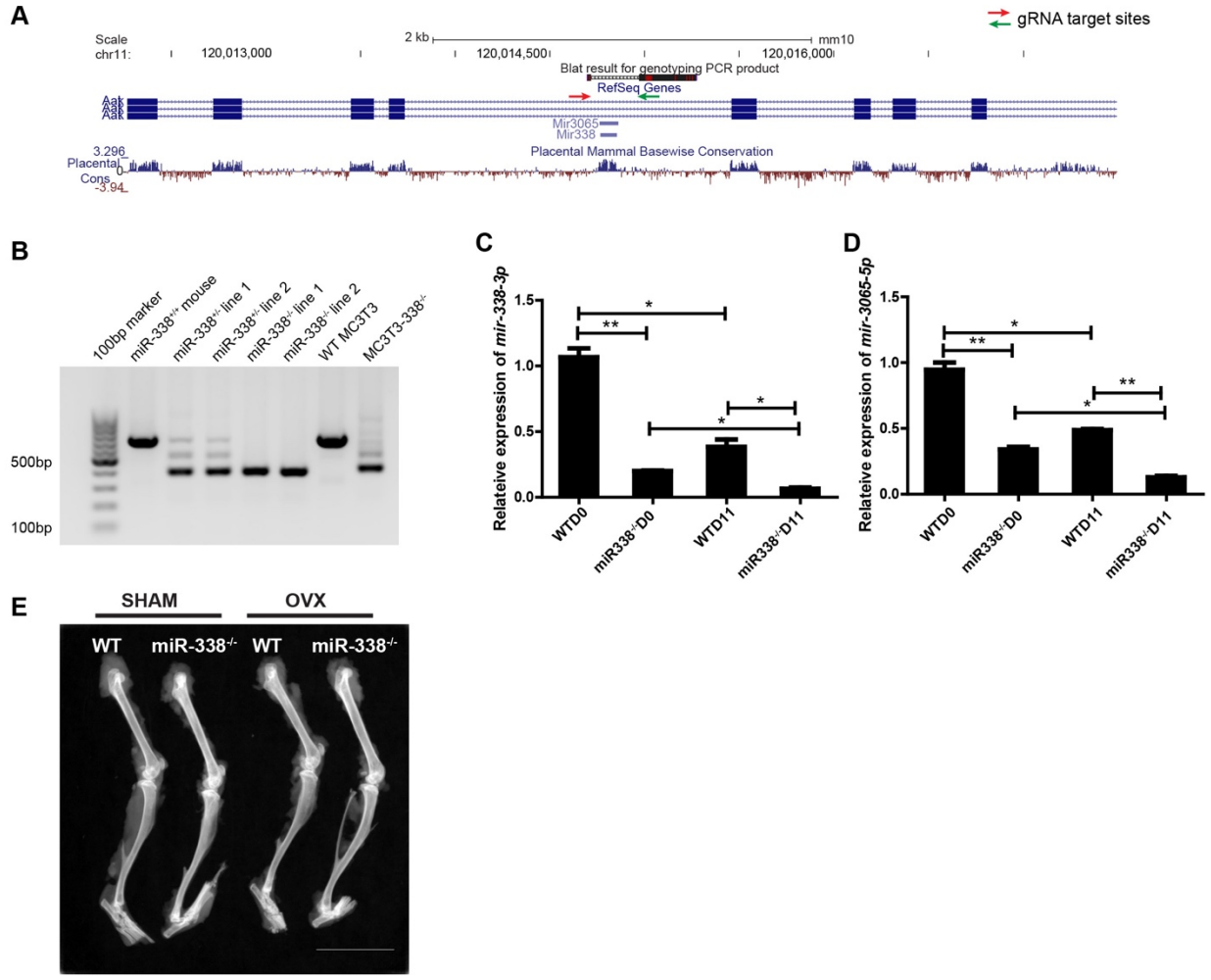


Figure S6 Design of CRISPR-Cas9 mediated knockout of miR-338 cluster in mouse. (A) Genomic location of gRNAs for knockout of miR-338 cluster. **(B)** Representative genotyping for miR-338 cluster knockout mice and MC3T3-E1, noted the residual bands in MC3T3-338$^{-/-}$ cells indicating incomplete knockout of miR-338 cluster caused by lentivirus method. Expression level of miR-338-3p **(C)** and miR-3065-5p **(D)** in the BMSCs isolated from wildtype (WT) and miR-338$^{-/-}$ mice. **(E)** Representative X-ray images of the femurs isolated from different groups of mice. (Scale bar = 1cm) (n=3 per group, t-test , *p<0.05, ** p<0.01)

Figure S7

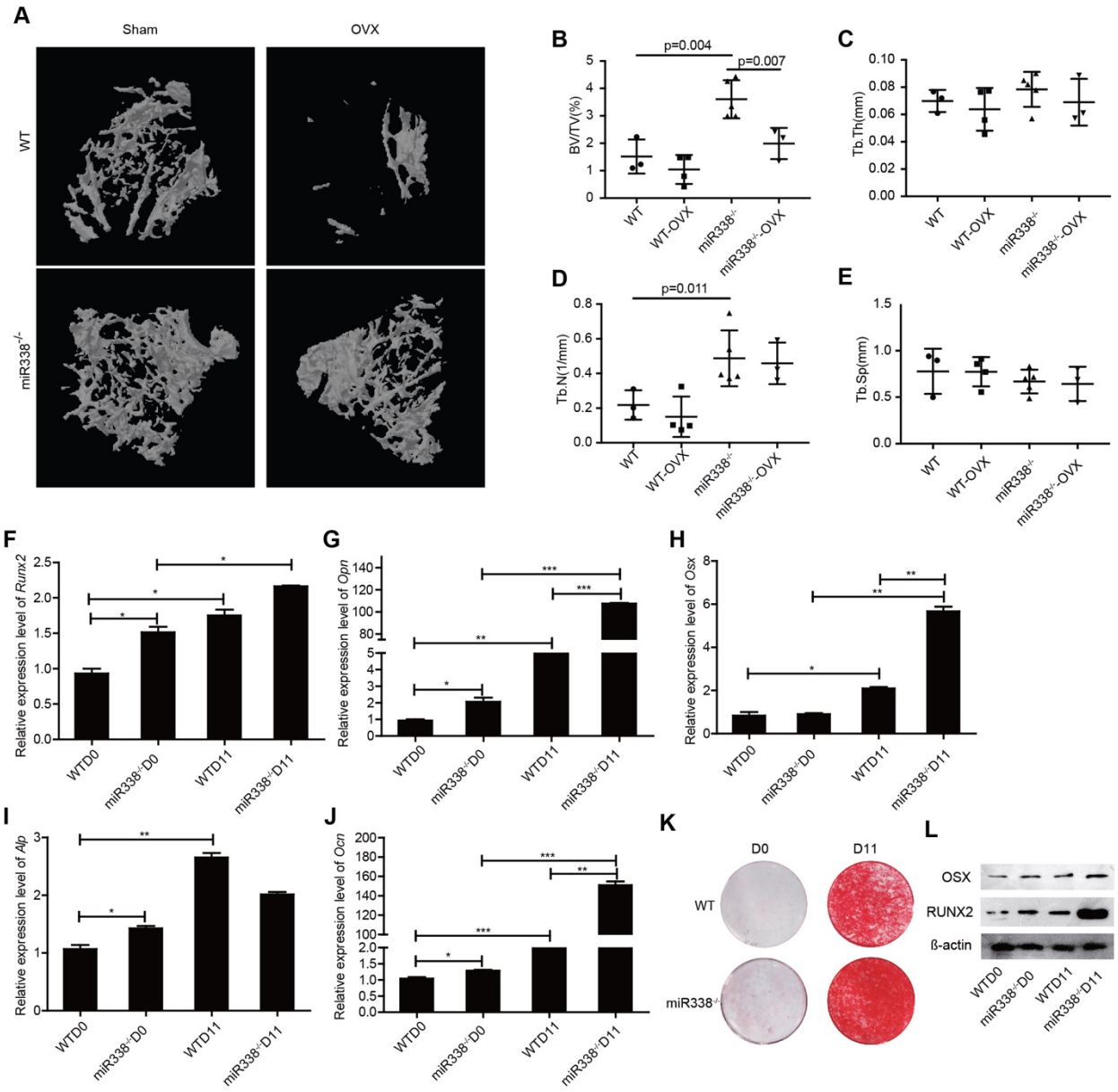


Figure S7 Loss of the miR-338 cluster resulted in insusceptibility to osteoporosis (A) Representative micro-CT results in the same position above the growth plate from different femurs isolated from different groups of mouse. Bone volume fraction (BV/TV) **(B)**, Trabecular Thickness (Tb.Th) **(C)**, average trabecular number (Tb.N) **(D)** and trabecular spacing (Tb.Sp) **(E)** in femurs. (n=3 for WT (wildtype) and and miR-338$^{-/-}$-OVX (miR-338$^{-/-}$ mice 16 weeks after ovariectomy surgery), n=4 for WT-OVX (wildtype mice 16 weeks after ovariectomy surgery) and miR-338$^{-/-}$) (t-test). qRT-PCR showing expression level of Runx2 **(F)**, Opn **(G)**, Osx **(H)**, Alp **(I)**, and Ocn **(J)** in the BMSCs isolated from WT and miR-338$^{-/-}$ femurs without (D0) or with osteoblastic induction (D11). (n=3 per group, t-test, *p<0.05, ** p<0.01, *** p<0.001). **(K)** Representative alizarin red staining showing mineralization nodes generated by BMSCs isolated from WT and miR-338$^{-/-}$ mice treated with osteoblastic induction medium. **(L)** Western Blot showing expression change of OSX and RUNX2 in different group.

Figure S8

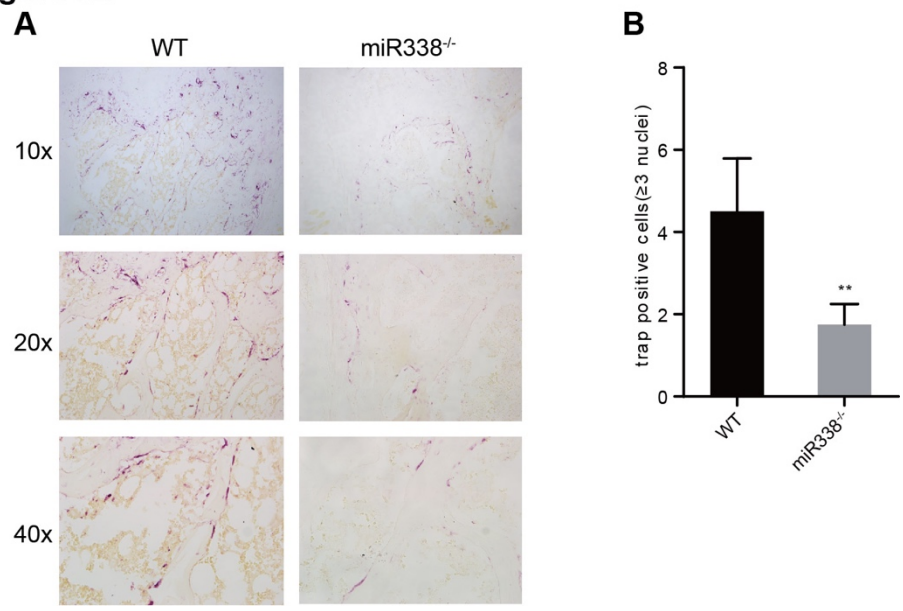
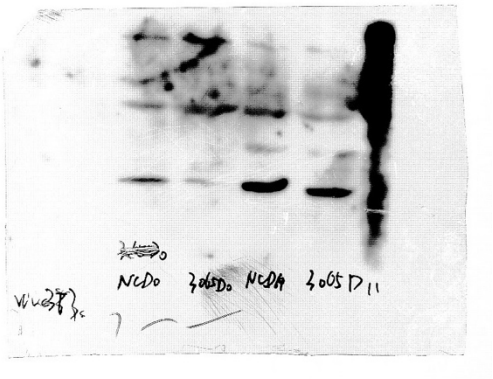


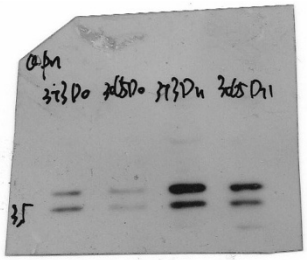
Figure S8 Decrease of number of osteoclasts in miR-338$^{-/-}$ mice. (A) Representative trap staining of femurs sections collected from wildtype (WT) and miR-338$^{-/-}$ mice. **(B)** Count of osteoclast cells with more than 3 nuclei. (n=6 visons from 3 femurs per group, t-test, *p<0.05, ** p<0.01).

Fig1G.Raw data

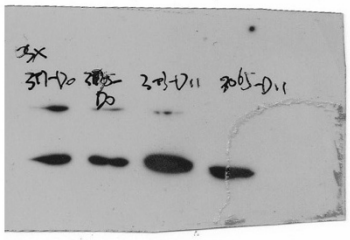
RUNX2



OPN



OSX



b-ACTIN

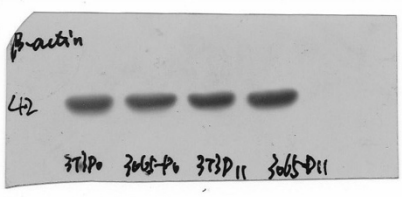
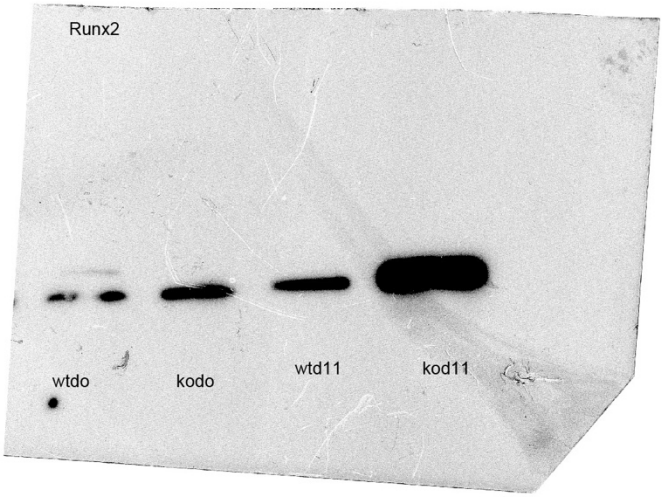
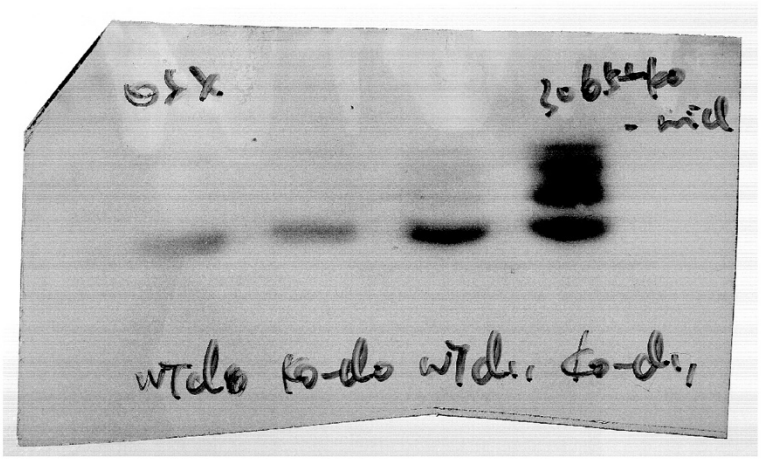


Fig8L.Raw data

Runx2



OSX



b-ACTIN