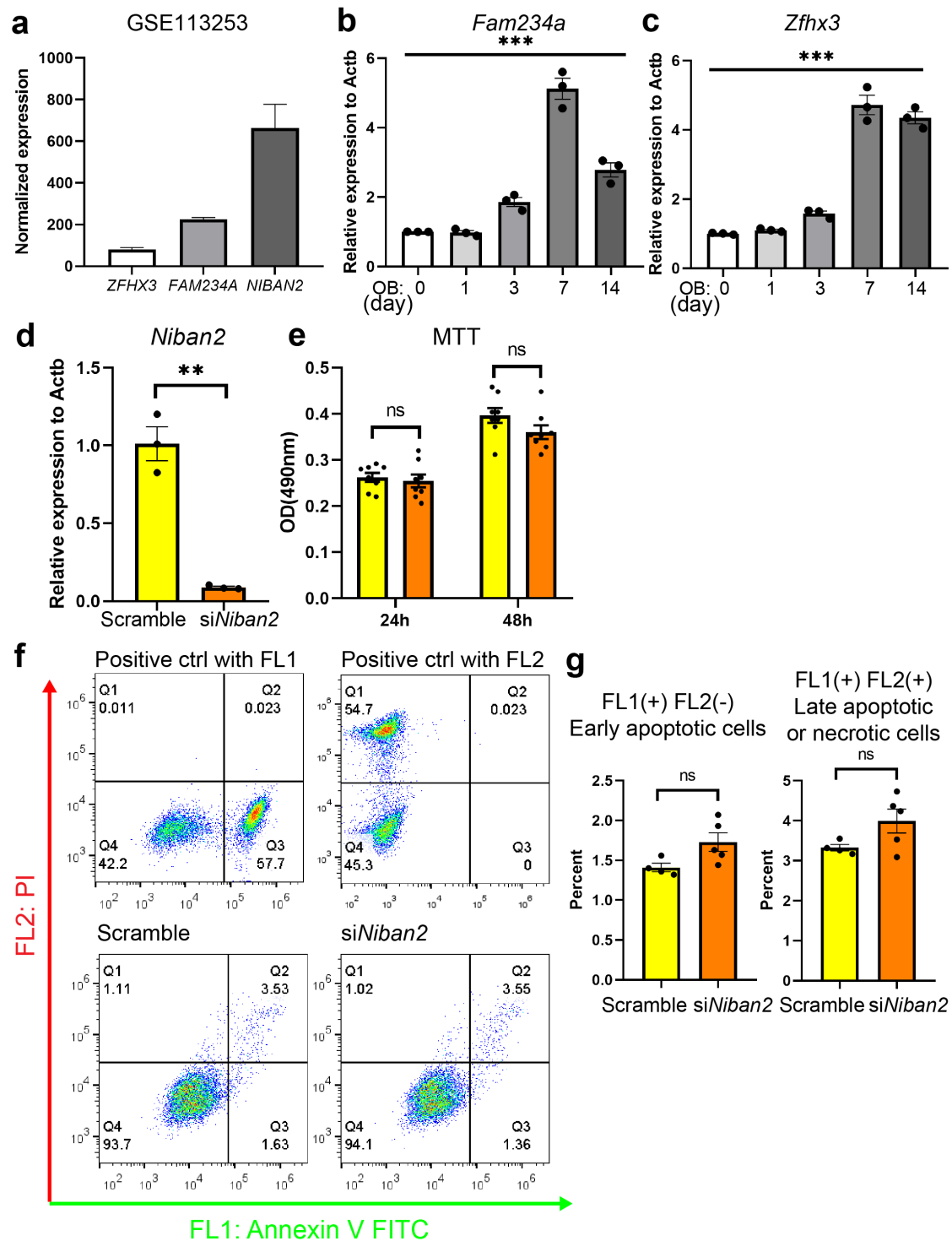




1

2 **Figure S1.** (a) *ZFH3*, *FAM234A*, and *NIBAN2* mRNA abundance in OBs derived from

3 human mesenchymal stem cells. Relative expression of *Fam234a* (b) and *Zfhx3* (c)

4 during osteoblast differentiation. (d) Result of quantitative RT–PCR to detect relative

5 expression of *Niban2* in *Niban2* knockdown and the control MC3T3-E1. (e) Cell

6 growth and viability detection with MTT in *Niban2* knockdown and the control
7 MC3T3-E1. (f) Representative detection of apoptosis by flow cytometry with Annexin
8 V and PI staining in *Niban2* knockdown and the control MC3T3-E1. (g) Quantitative
9 analysis of the apoptosis in panel e. Data are presented as the mean $\pm$ SEM. *** $P <$
10 0.001 tested by one-way ANOVA followed by Bonferroni's post hoc test. Repeats
11 information and source data are provided in the Source Data File.

12

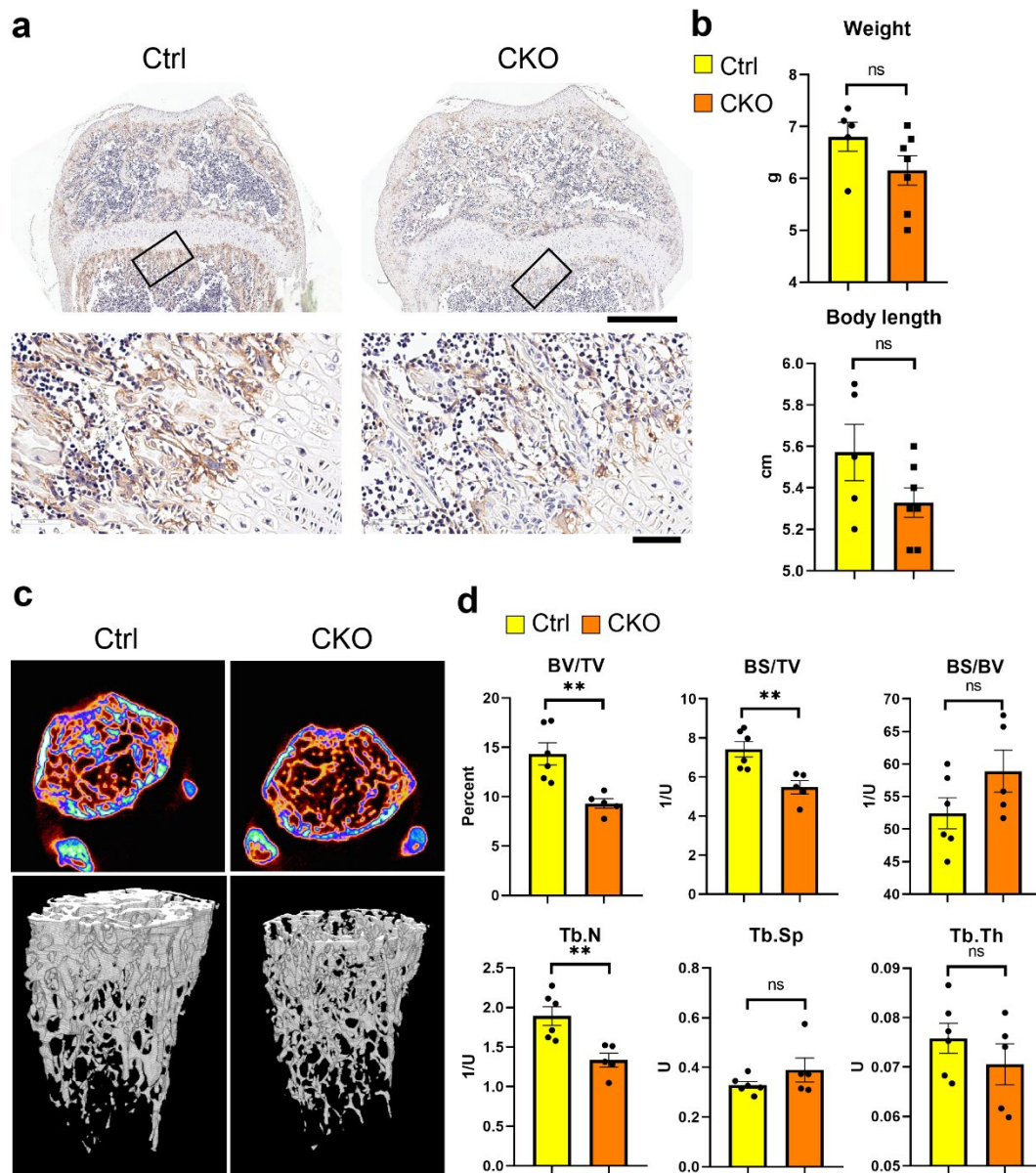


Figure S2. (a) Immunohistochemistry of Niban2 expressions in *Niban2* CKO mice and its control in 4 week (scale bar: 500 μ m; for magnification, scale bar: 50 μ m). Representative images for 3 independent samples. (b) Weight and body length of *Niban2* CKO mice and its control. (c) Representative cross section and reconstruction images of *Niban2* CKO mice and control mice in 8 weeks by μ CT. (d) Quantitative analysis of the μ CT results of panel a. Data are presented as the mean $\pm$ SEM. * P < 0.05 versus the control; ** P < 0.01 versus the control tested by unpaired Student's t test. Repeats information and source data are provided in the Source Data File.

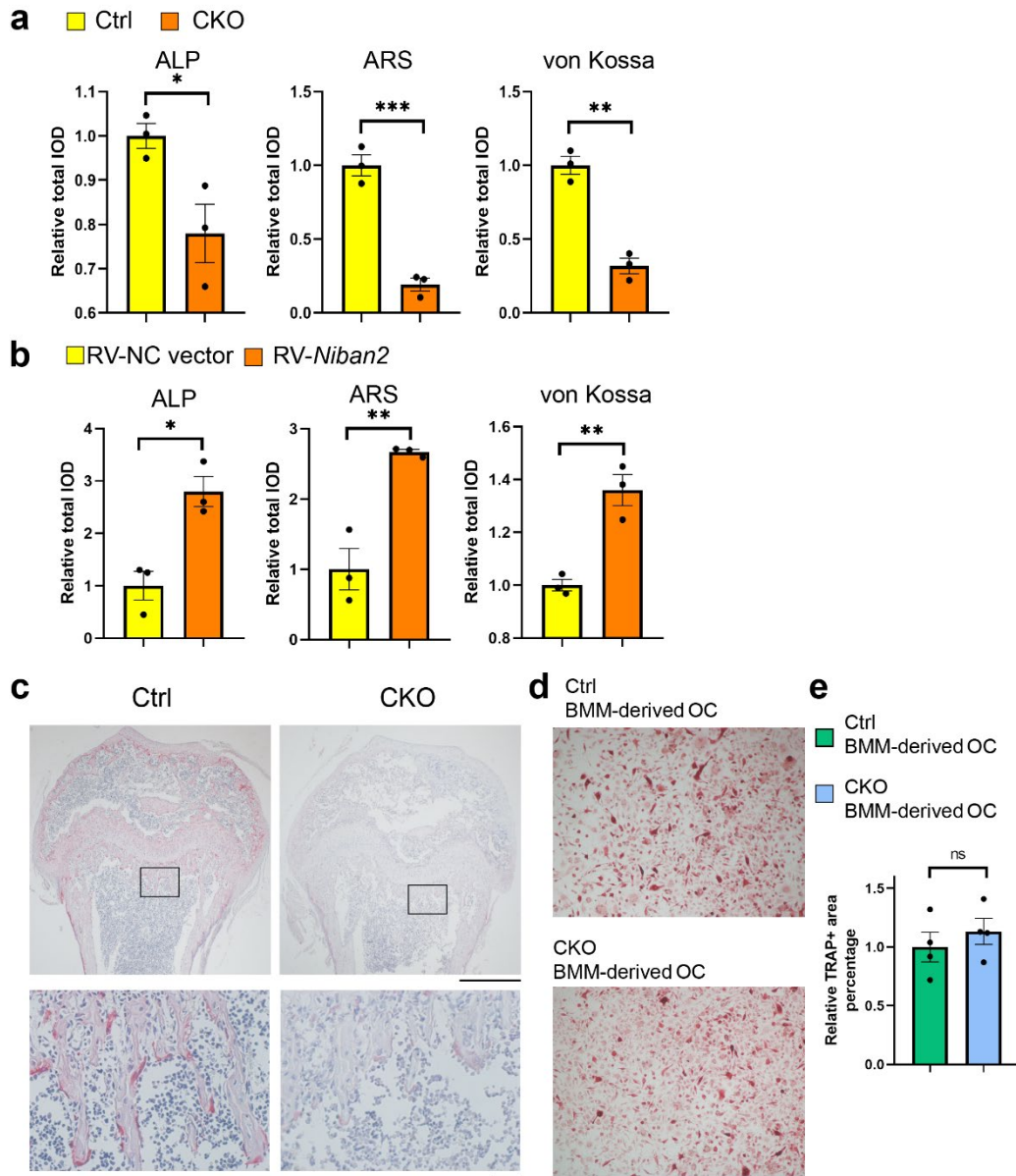


Figure S3. (a-b) Quantitative analysis of the osteoblast differentiation staining of Figure 4b. (c) Representative TRAP staining of *Niban2* CKO mice and control mice (scale bar: 800 μ m; for magnification, scale bar: 50 μ m). (d) Representative TRAP staining of BMM-derived osteoclasts from *Niban2* CKO mice and control mice *in vitro* (scale bar: 50 μ m). (e) Quantitative analysis of the TRAP staining in panel e. Data are presented as the mean $\pm$ SEM. * P < 0.05; ** P < 0.01; *** P < 0.001 tested by one-way ANOVA followed by unpaired Student's *t* test and Bonferroni's post hoc test. Repeats information and source data are provided in the Source Data File.

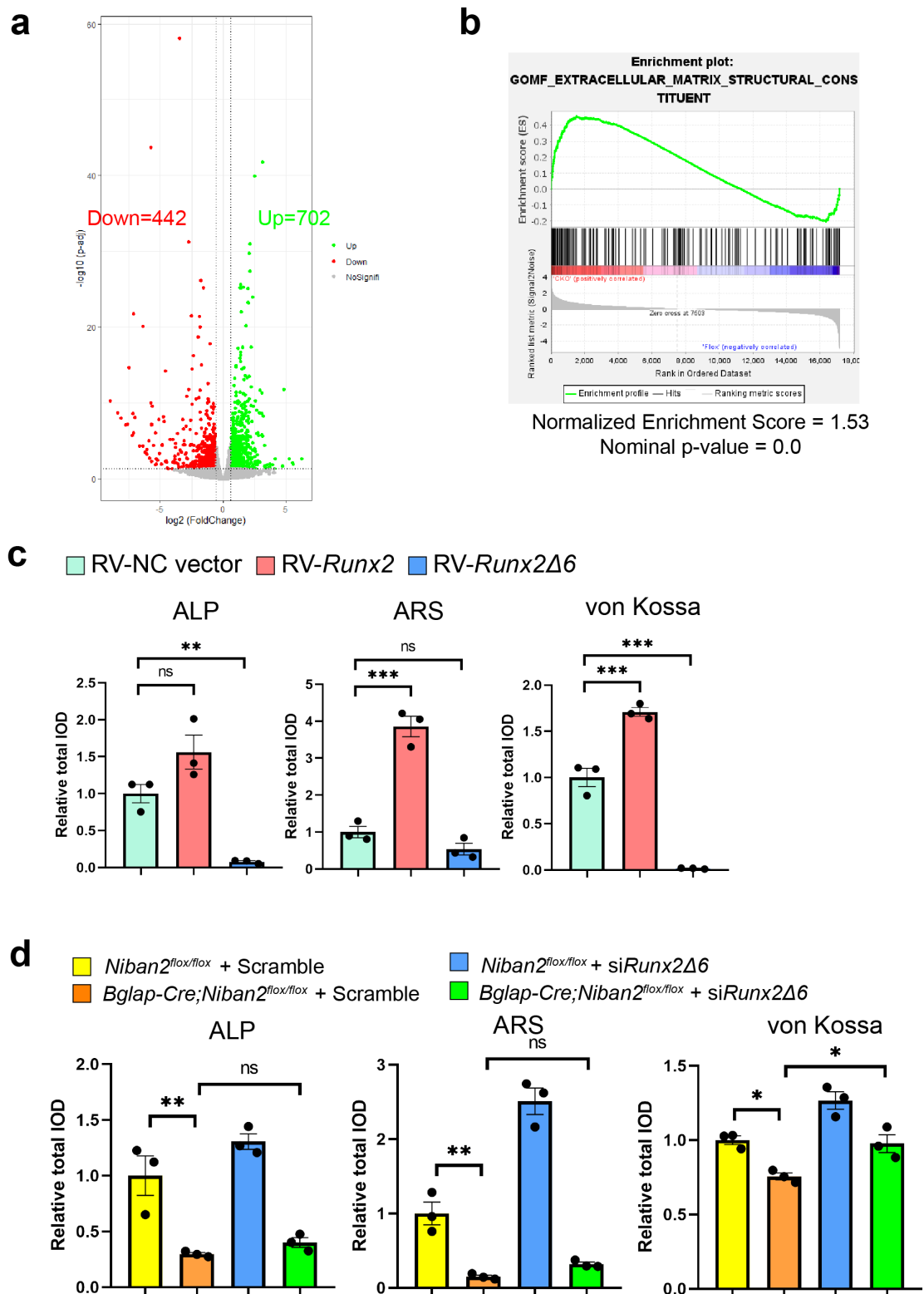


Figure S4. (a) Volcano plot of the transcriptome data of *Niban2* CKO pre-OBs and the controls. DEGs were marked green or red for downregulated and upregulated, respectively. (b) GSEA result of extracellular matrix formation in *Niban2* CKO pre-

32 OBs and control cells. (c) Quantitative analysis of the osteoblast differentiation staining
33 of Figure 5g. (c) Quantitative analysis of the osteoblast differentiation staining of Figure
34 5g. (d) Quantitative analysis of the osteoblast differentiation staining of Figure 5i. Data
35 are presented as the mean $\pm$ SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ tested by one-
36 way ANOVA followed by unpaired Student's t test and Bonferroni's post hoc test.
37 Repeats information and source data are provided in the Source Data File.
38

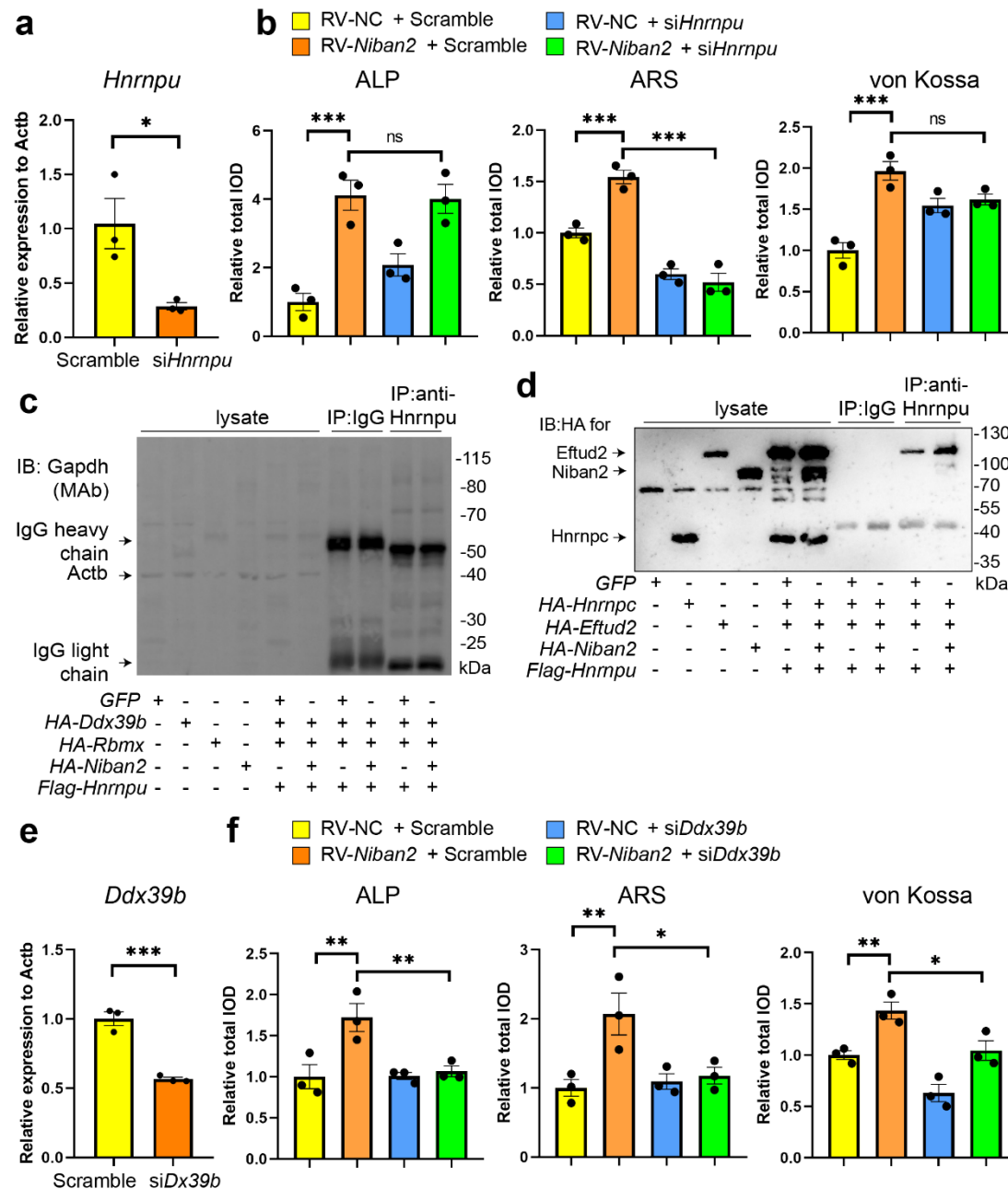


Figure S5. (a) Result of quantitative RT-PCR to detect relative expression of *Hnrnpu* in *Hnrnpu* knockdown pre-OBs and its control cells. (b) Quantitative analysis of the osteoblast differentiation staining of Figure 6m. (c) Western blots of mouse anti-Actb with the same blot of Figure 7g. Representative images for 3 independent repeats. HEK293T cells were first transfected with plasmids expressing HA-labeled spliceosomal complex components. IB was carried out to detect Niban2 and the other

spliceosomal complex components, including Hnrnpc and Eftud2 (d). Representative images for 3 independent repeats. (e) Result of quantitative RT–PCR to detect relative expression of *Ddx39b* in *Ddx39b* knockdown cells and the control cells. (f) Quantitative analysis of the osteoblast differentiation staining of Figure 7j. Data are presented as the mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ tested by one-way ANOVA followed by unpaired Student's t test and Bonferroni's post hoc test. Repeats information and source data are provided in the Source Data File.

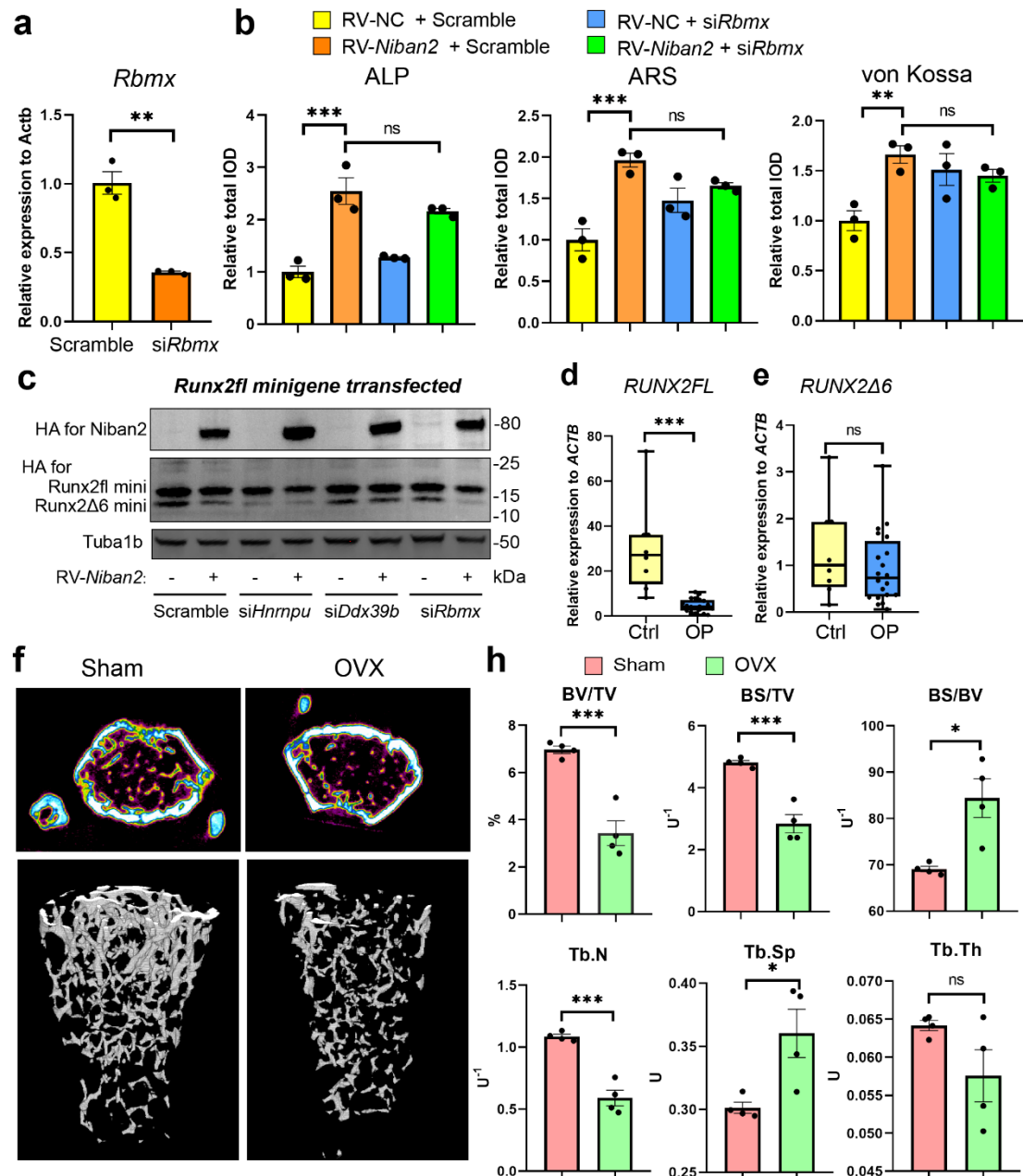


Figure S6. (a) Result of quantitative RT-PCR to detect relative expression of *RbmX* in *RbmX* knockdown cells and the control cells. (b) Quantitative analysis of the osteoblast differentiation staining of Figure 7l. (c) Western blots of HA for Niban2 and Runx2 mini in *Niban2*-OE and its controls with transfection of *siHnrnpu*, *siDdx39b*, *siRbmX*, and scramble in *Runx2* mini expressing MC3T3-E1. Relative expression of *RUNX2FL* (d) and *RUNX2Δ6* (e) to *ACTB* in osteoporosis patients and controls, respectively. (f) Representative cross section and reconstruction images of ovariectomy mice and

62 control mice by μ CT. (f) Quantitative analysis of the μ CT results of panel a. Data are
63 presented as the mean $\pm$ SEM. $*P < 0.05$ versus the control; $**P < 0.01$ versus the
64 control; $***P < 0.001$ tested by unpaired Student's t test. Repeats information and
65 source data are provided in the Source Data File.

	Forward (5'-3')	Reverse (5'-3')
Primers for qPCR		
<i>Mus musculus</i>		
<i>Actb</i>	GCGGGCGACGATGCT	CACGGTTGGCCTTAGGGTT
<i>Niban2</i>	CCCTCTCATCCTGTGGCATC	CGAACGCAATCCTGAAGCAC
<i>Zfhx</i>	CAAACCCAGTAGCATGGAGCA	ATGTAGGTCTGGAGGCTGGA
<i>Fam234a</i>	AGATCCTGTGCTGATGAAGGC	TACAAGGGCGACATCTTGGG
<i>Hnrnpu</i>	TAGAGGCCGTGGAGGATTCA	GGCCACGTGGGTATGGATAG
<i>Ddx39b</i>	GAGGTCCAGCATGAGTGCAT	TGTGACACATCACCAGCACA
<i>RbmX</i>	CACGTAGTGCTCCACCTACA	ATCAGATCGGCTTCCTCCAC
<i>Runx2-P1</i>	CATGGCCGGGAATGATGAGA	GGCCACTTGGGGAGGATTTG
<i>Runx2-P2</i>	GAACCAAGAAATCCCAGGCAGG	GTTGGAGAAGCGGCTCTCAG
<i>Runx2-P3 (exon 6)</i>	TCTGACCGCCTCAGTGATTT	AAGGACTTGGTGCAGAGTTCA
<i>Homo sapiens</i>		
<i>ACTB</i>	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
<i>NIBAN2</i>	CTGACGGAGTTCCTCCAGTTC	GAGGTTCCCCGAGAAGACGAT
<i>RUNX2Δ6</i>	GAACCAAGAAATCCCAGGCAGG	GTTGGAGAAGCGGCTCTCAG
<i>RUNX2FL (exon 6)</i>	TCTGACCGCCTCAGTGATTT	AAGGACTTGGTGCAGAGTTCA
Primers for RIP-qPCR		
<i>ACTB exon 4</i>	AGGGAAATCGTGCGTGACAT	GGAAAAGAGCCTCAGGGCAT
<i>RUNX2 exon 6</i>	TCTGACCGCCTCAGTGATTT	AAGGACTTGGTGCAGAGTTCA
siRNA		
<i>siNiban2</i>	CUUACGCCCCGACACUAUUATT	UAAUAGUGUCGGGCGUAAGTT
<i>siRunx2Δ6</i>	CGGGAACCAAGAAAUCCATT	UGGGAUUUCUUGGUUCCCGTT
<i>siHnrnpu</i>	GUUGCUUGGUGAAGAAGAATT	UUCUUCUUCACCAAGCAACTT
<i>siDdx39b</i>	CUGGUGAUGUGUCACACUATT	UAGUGUGACACAUCACCAGTT
<i>siRbmX</i>	GUCUAUCAAGAAGGACGAATT	UUCGUCCUUCUUGAUAGACTT

66

67 **Table S1** Oligos used in this research.