Fig. S1. Articular chondrocytes with FoxO1 deficiency exhibited reduced expression of anabolic genes and upregulation of catabolic markers. (A) Real-time qPCR analyses of gene expression from Ad-Con (Con) and Ad-Cre (Cre) transduced *FoxO1*$^{f/f}$ primary articular chondrocytes. The mRNA abundance was normalized to that of the gene β-actin and then normalized to the Ad-Con transduced cells. Data are means ± SD. *$p < 0.05$. N = 3.

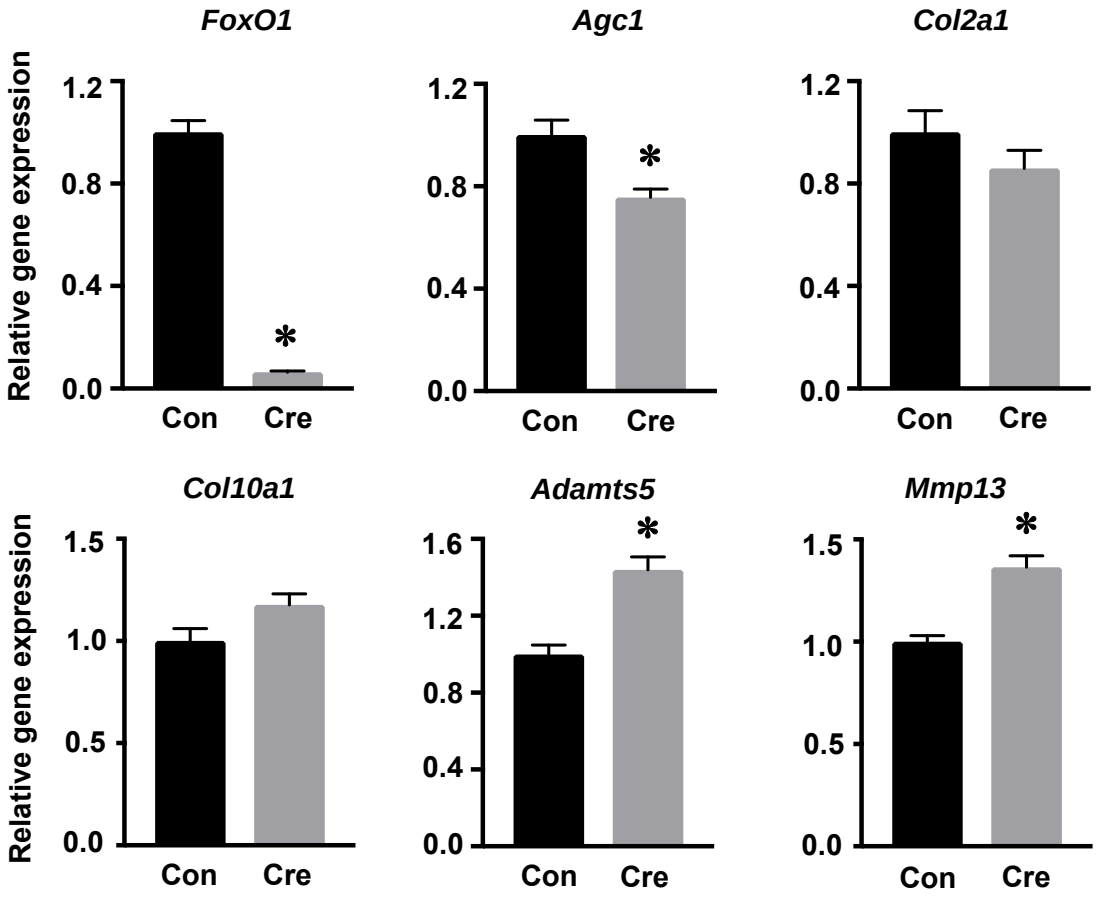

Fig. S2. FoxO1 LOF mice display abnormal collage organization and an overall decrease of stained area in superficial zone of FoxO1 LOF articular cartilage. Picrosirius red stained sections of control (*FoxO1*$^{f/f}$; Con) and *FoxO1*^{Agc1ER} (*Agc1Cre*^{ERT2}*;FoxO1*$^{f/f}$; Mut) mice at 4 months of age under polarized light. All mice, including Cre negative controls, received tamoxifen. Intensity of stained area in superficial zone of cartilage on tibial plateau of knee sections at 4 months. All results were compared to Cre negative controls and expressed as means ± SD. *$p < 0.05$. N = 5. Scale bar, 100 μm.

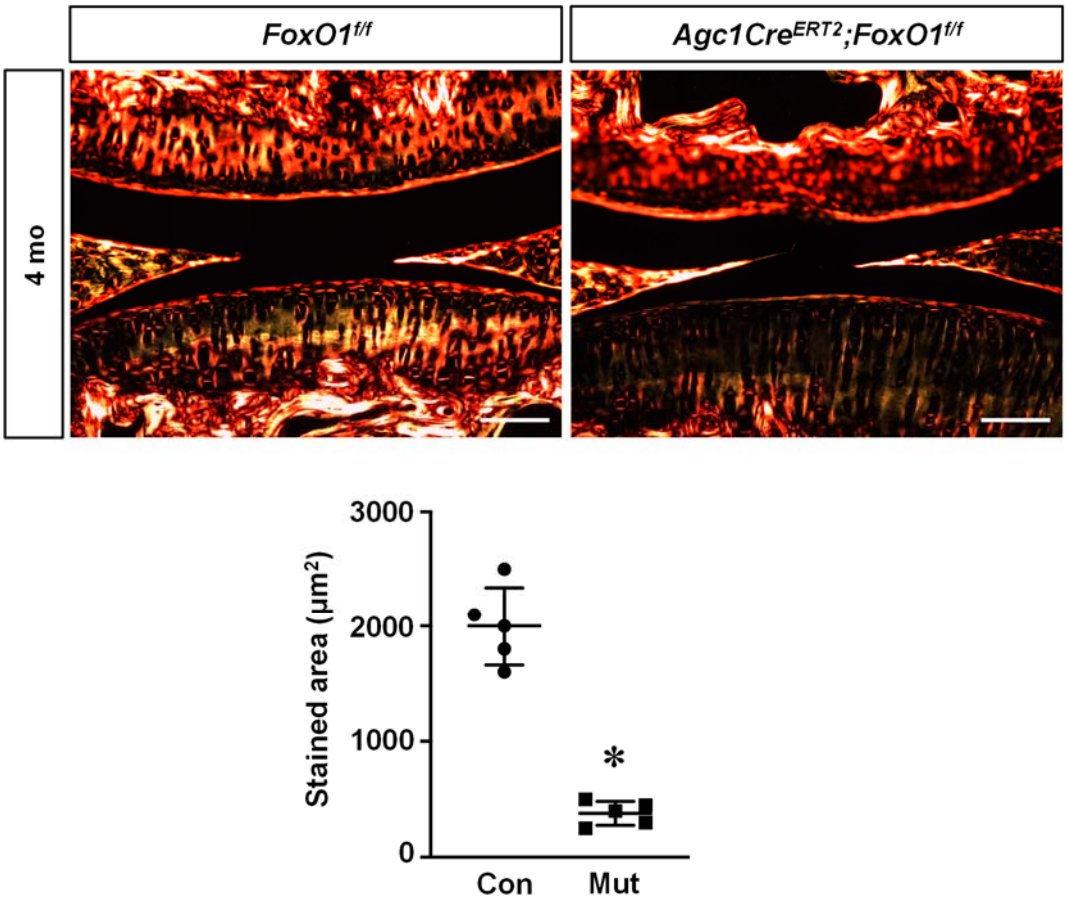

Fig. S3. FoxO1 LOF mice display an overall loss of bone mass in their subchondral plates. Micro-CT assessment and quantification of BV/TV of subchondral bone plate underneath the tibial plateau from 4- and 7-month-old mice. Data are means ± SD. *p < 0.05 compared to controls. N = 5. Scale bar, 200 μm.

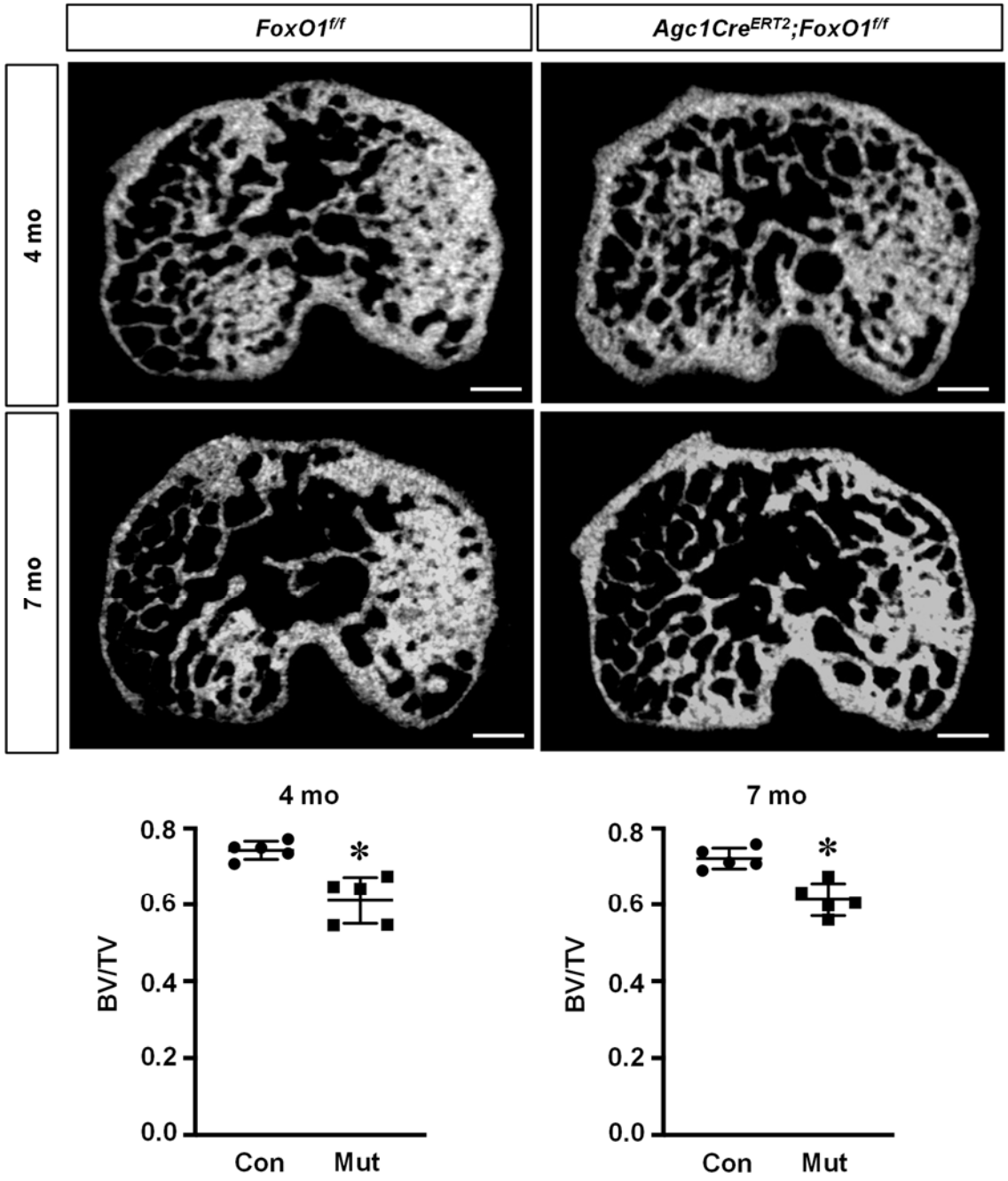

Fig. S4. FoxO1 GOF mice exhibit less synovial tissue hyperplasia at 12 weeks following MLI. Safranin O/Fast Green staining of knee sections of control (*Rosa-FoxO1$^{f/f}$*) and *Rosa-FoxO1^{Agc1ER}* (*Agc1Cre^{ERT2};Rosa-FoxO1$^{f/f}$*) mice at 12 weeks following MLI injury. Arrows indicate synovial tissue hyperplasia. N = 5. Scale bar, 100 μm.

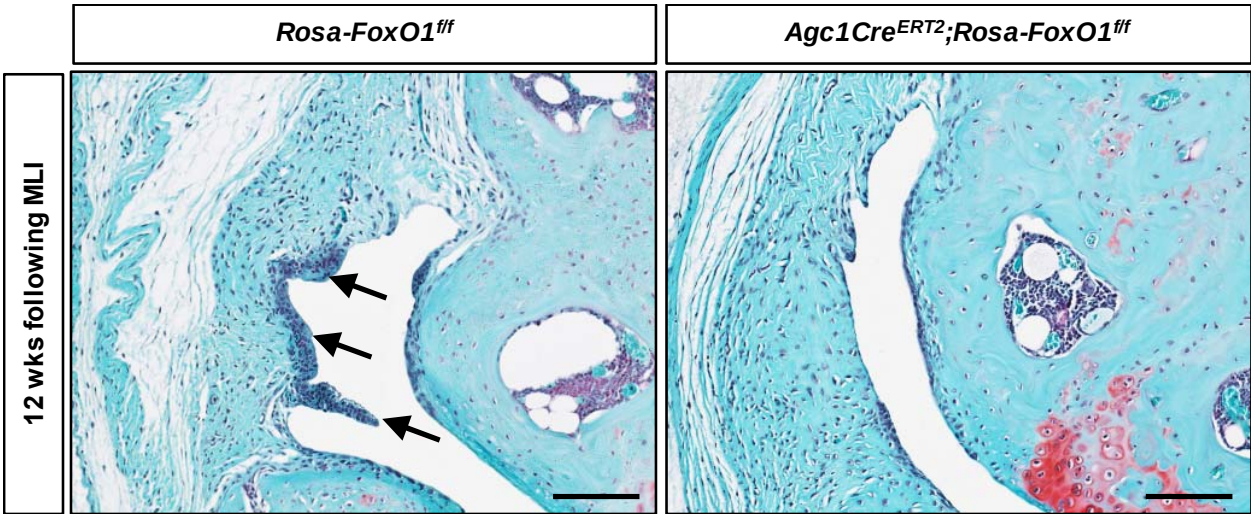

Fig. S5. Induction of FoxO1 in cartilage prior to MLI attenuates MLI-associated subchondral sclerosis. Micro-CT assessment and quantification of BV/TV of subchondral bone plate at 8 and 12 weeks following MLI. Data are means ± SD. *p < 0.05 compared to controls. N = 5. Scale bar, 200 μm.

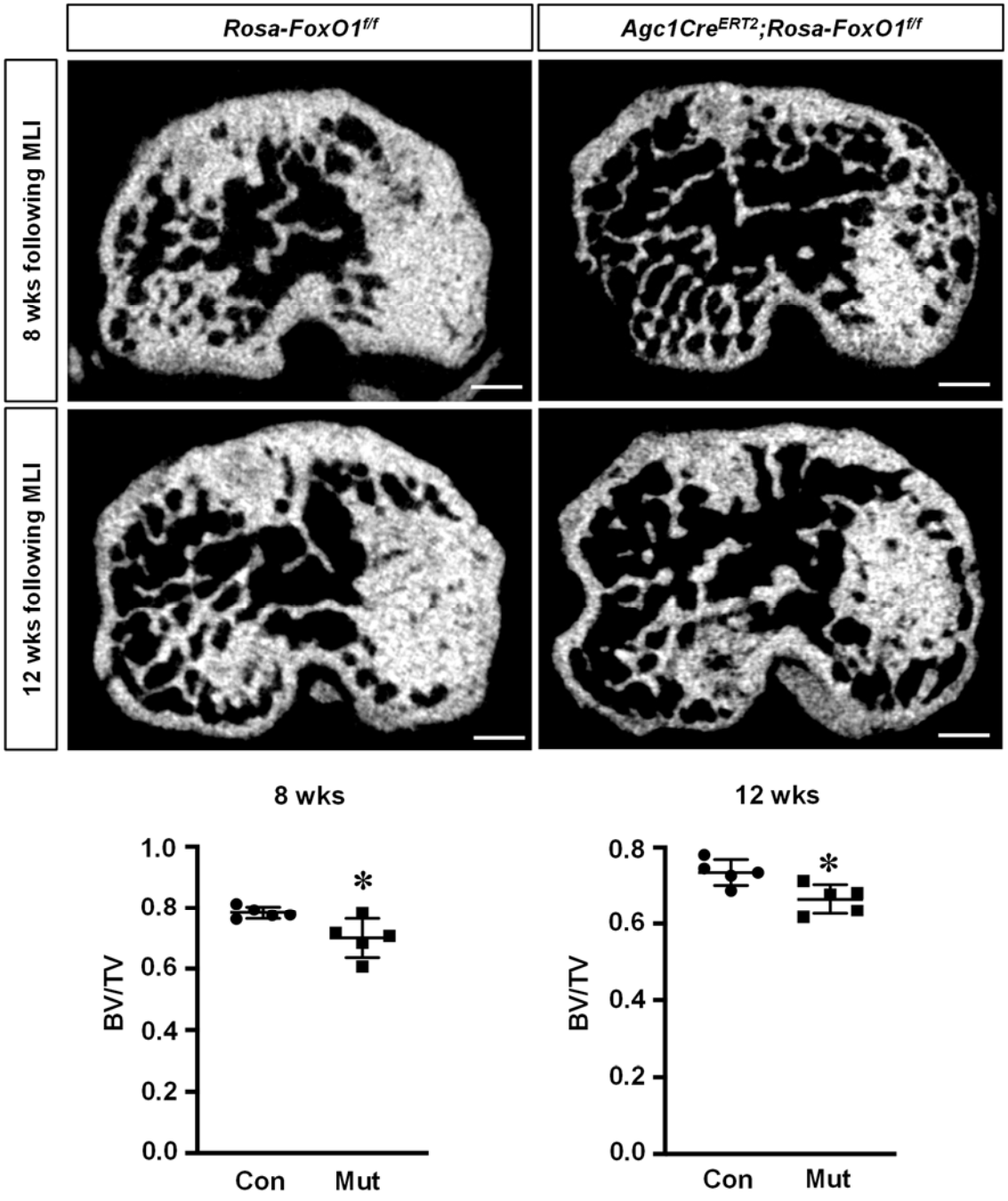

Fig. S6. Overexpression of FoxO1 in chondrocytes following MLI protects against surgically-induced OA. Safranin O/Fast Green staining of knee sections of control (*Rosa-FoxO1*$^{f/f}$; Con) and *Rosa-FoxO1*^{Agc1ER} (*Agc1Cre*^{ERT2}*;Rosa-FoxoO1*$^{f/f}$; Mut) mice at 12 weeks following MLI injury. All mice, including Cre negative controls, received tamoxifen. OARSI scores for the medial tibial plateau and femoral condyle at 12 weeks following MLI injury. *$p < 0.05$ compared to controls. N = 5. Scale bar, 50 μm.

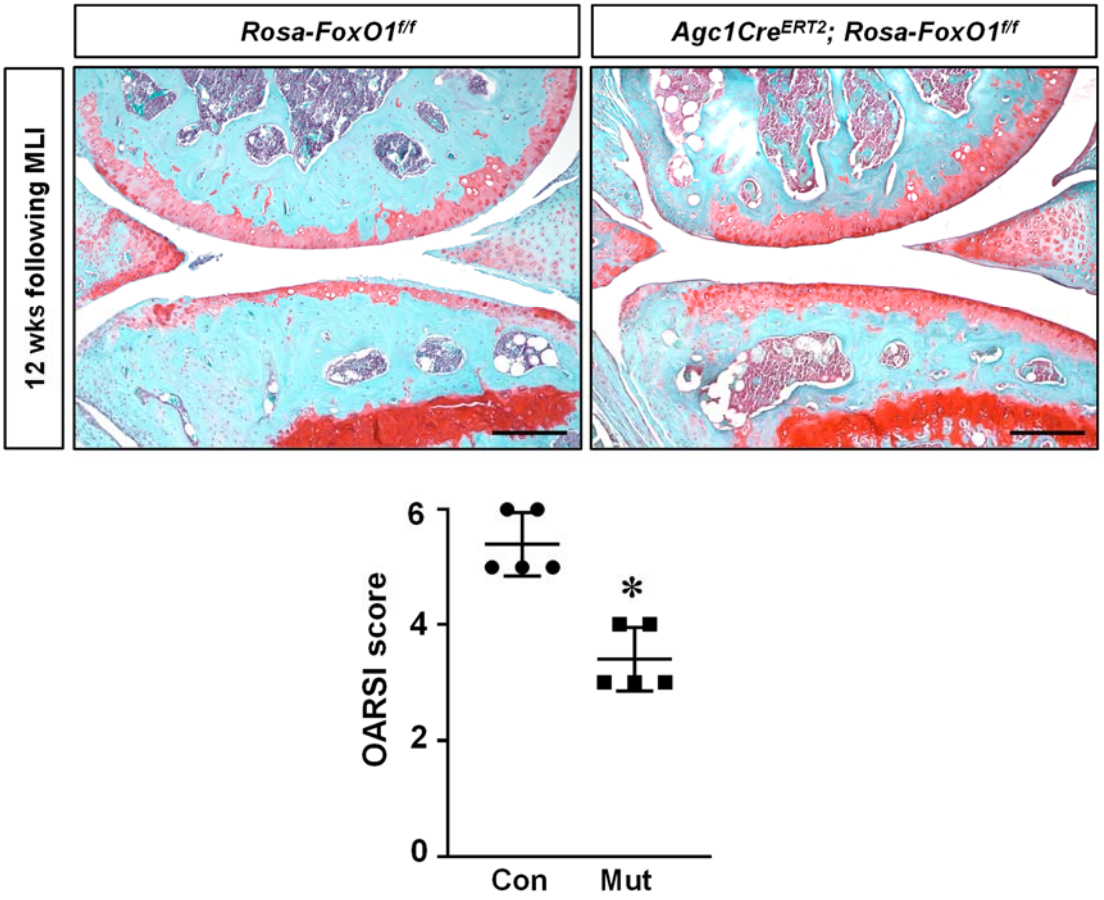

Fig. S7. Induction of FoxO1 in cartilage following MLI leads to less severity of MLI-associated subchondral sclerosis. Micro-CT assessment and quantification of BV/TV of subchondral bone plate at 12 weeks following MLI. Data are means ± SD. *p < 0.05 compared to controls. N = 5. Scale bar, 200 μm.

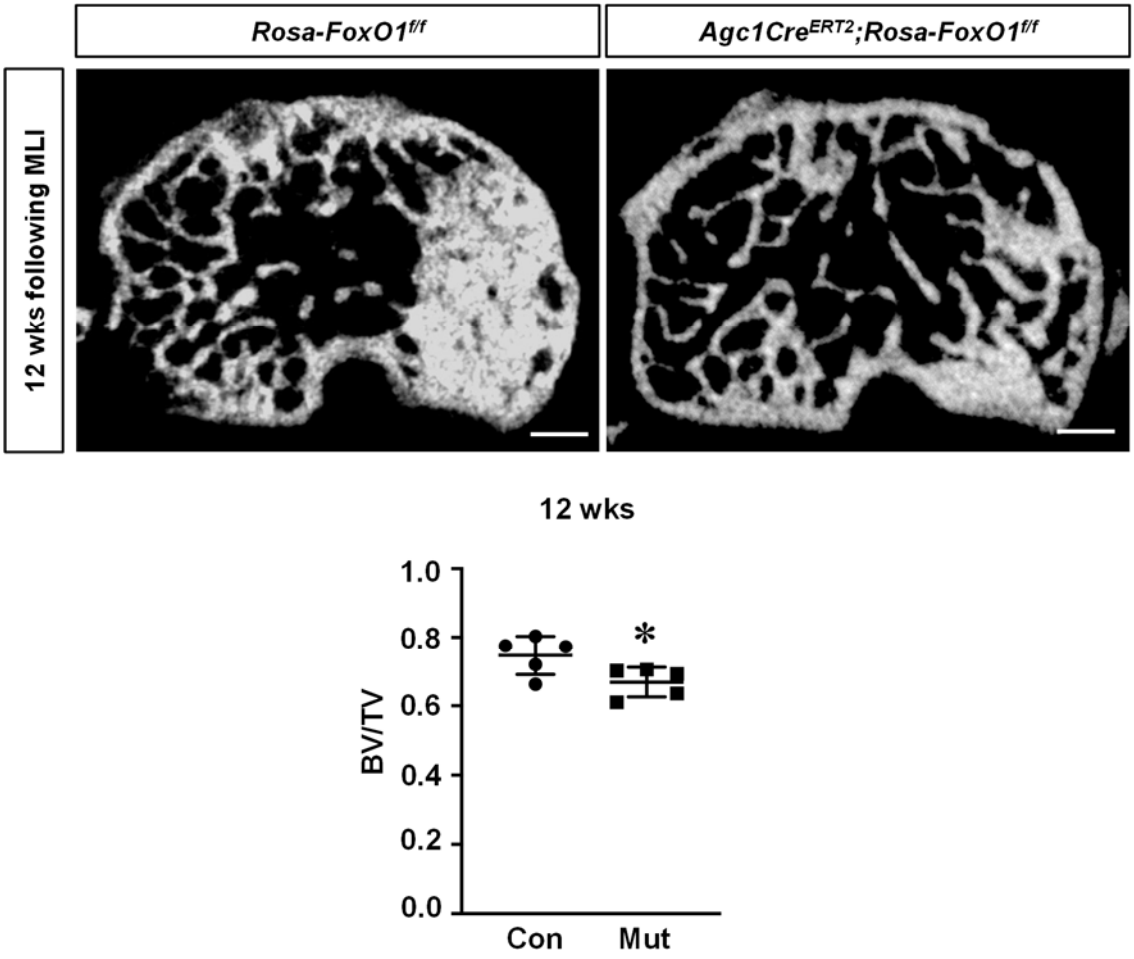

Fig. S8. Autophagy is constitutively active in articular chondrocytes. Western blot analyses of protein expression in primary articular chondrocytes treated with Bafilomycin (Baf) or DMSO (DMS) for 4 hours. Blots are representative of at least three independent experiments.

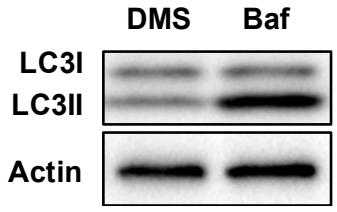

Fig. S9. FoxO1 binds to the promoters of autophagy-related genes. Standard PCR analyses for *Becn1* and *Map1lc3b* promoter regions containing the FoxO1 binding site following pull-down of genomic DNA from ATDC5 cells with IgG, H3 antibody, or FoxO1 antibody. N = 3.

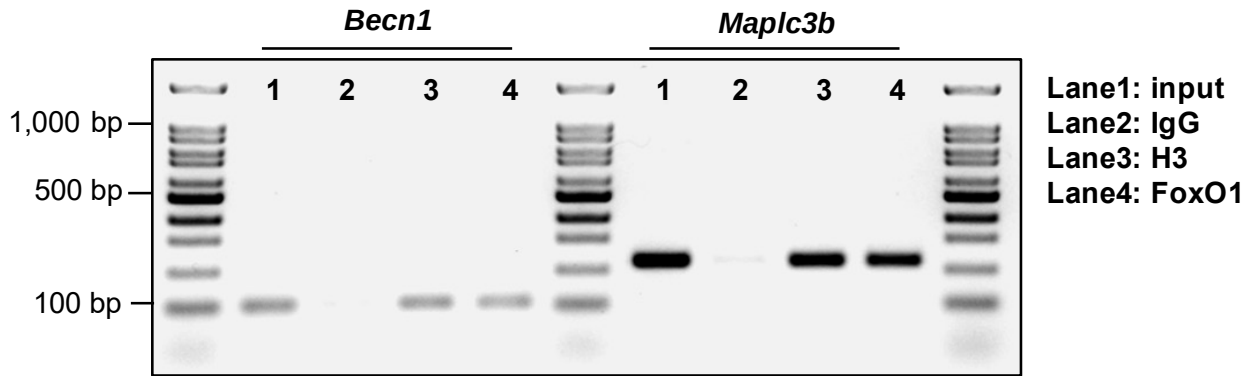

Fig. S10. FoxO1 LOF chondrocytes are shifted from a catabolic state to a more homeostatic state by rapamycin treatment. Real-time qPCR analyses of gene expression from *FoxO1*$^{f/f}$ articular chondrocytes transduced with Ad-Con or Ad-Cre and treated with rapamycin (Rapa) or DMSO (DMS) for the last 6 hours of the experiment. The mRNA abundance was normalized to that of the gene β-actin and then normalized to the Ad-Con transduced and DMSO treated cells. Data are means ± SD. *$p < 0.05$ compared to Ad-Con transduced cells or DMSO treated cells. N = 3.

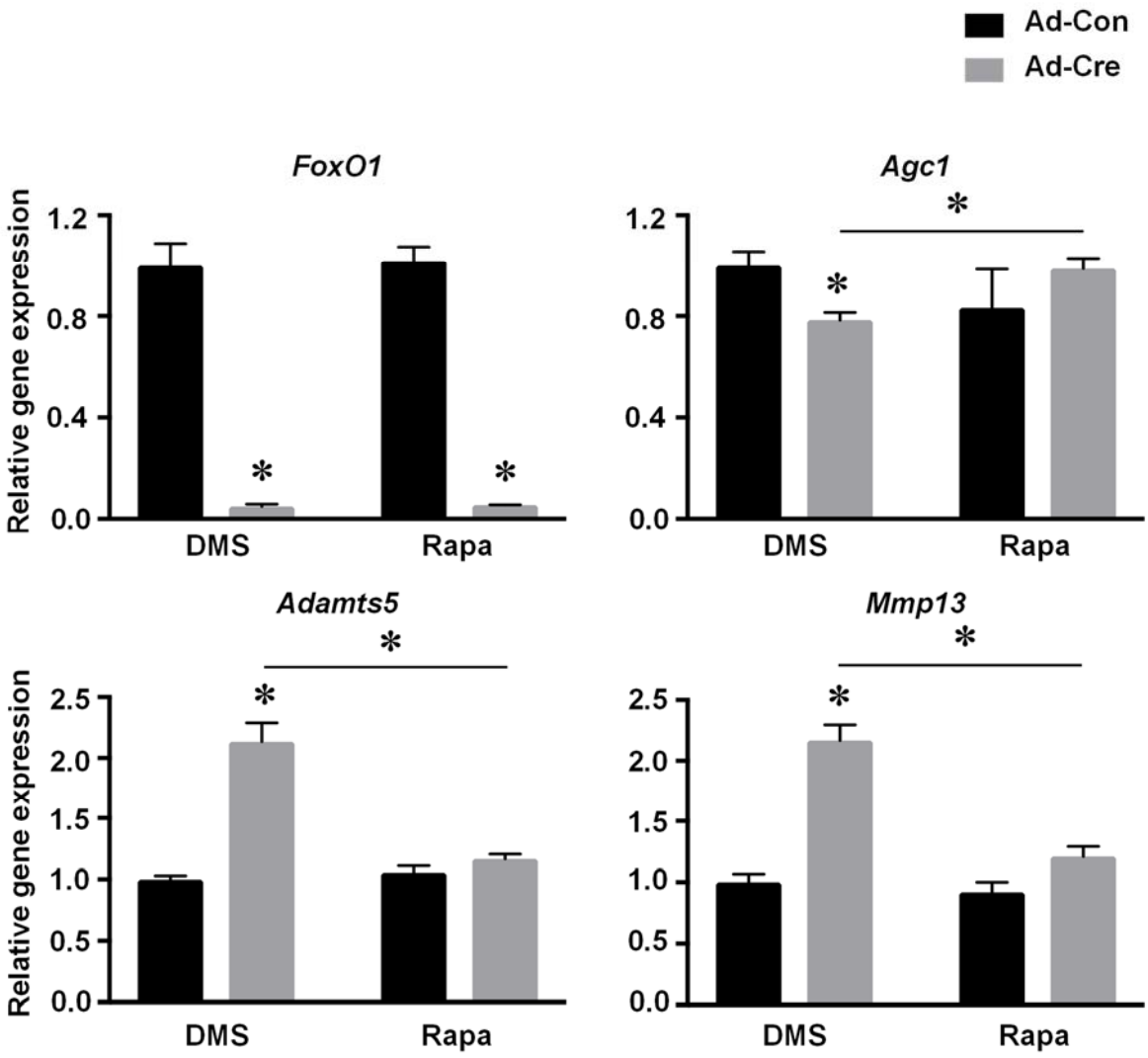

Fig. S11. Overexpression of FoxO1 in cartilage attenuates subchondral bone sclerosis caused by loss of TGFβ pathway. Micro-CT assessment and quantification of BV/TV of subchondral bone plate underneath the tibial plateau at 3 months. Data are means ± SD. *p < 0.05 compared to controls. N = 4. Scale bar, 200 μm.

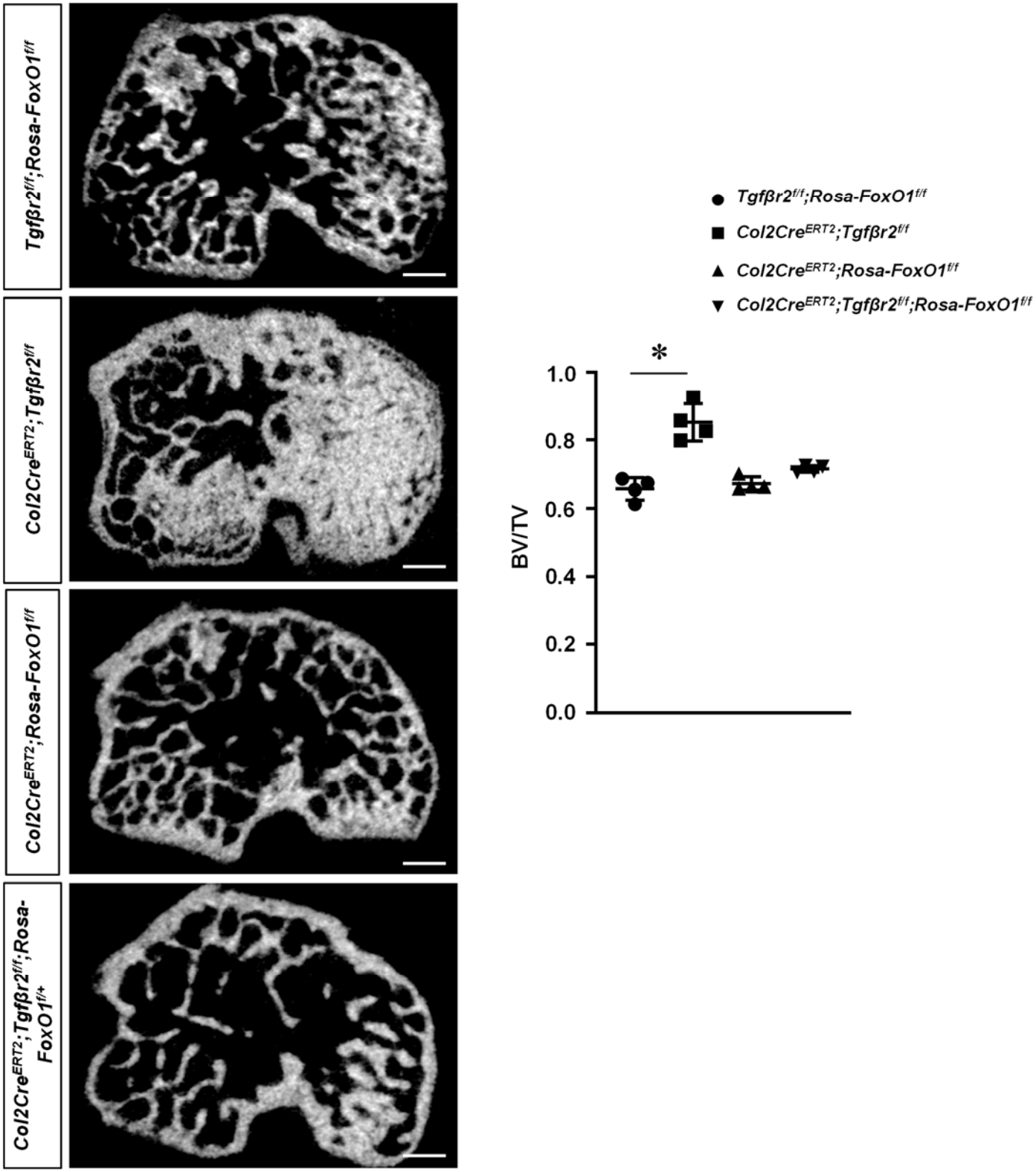

Fig. S12. Overexpression of FoxO1 in cartilage alters molecular responses to loss of TGFβ pathway. Immunohistochemical analyses for PCNA, Prg4, Col10A1, and Mmp13 on on knee sections of *Tgfβr2$^{f/f}$;Rosa-FoxO1$^{f/f}$*, *Col2Cre^{ERT2};Tgfβr2$^{f/f}$*, *Col2Cre^{ERT2};Rosa-FoxO1$^{f/f}$*, and *Col2Cre^{ERT2};Tgfβr2$^{f/f}$;Rosa-FoxO1$^{f/f}$* mice at 3 months of age. All mice, including Cre negative controls, received tamoxifen. N = 4. Scale bar, 100 μm.

Figure S12

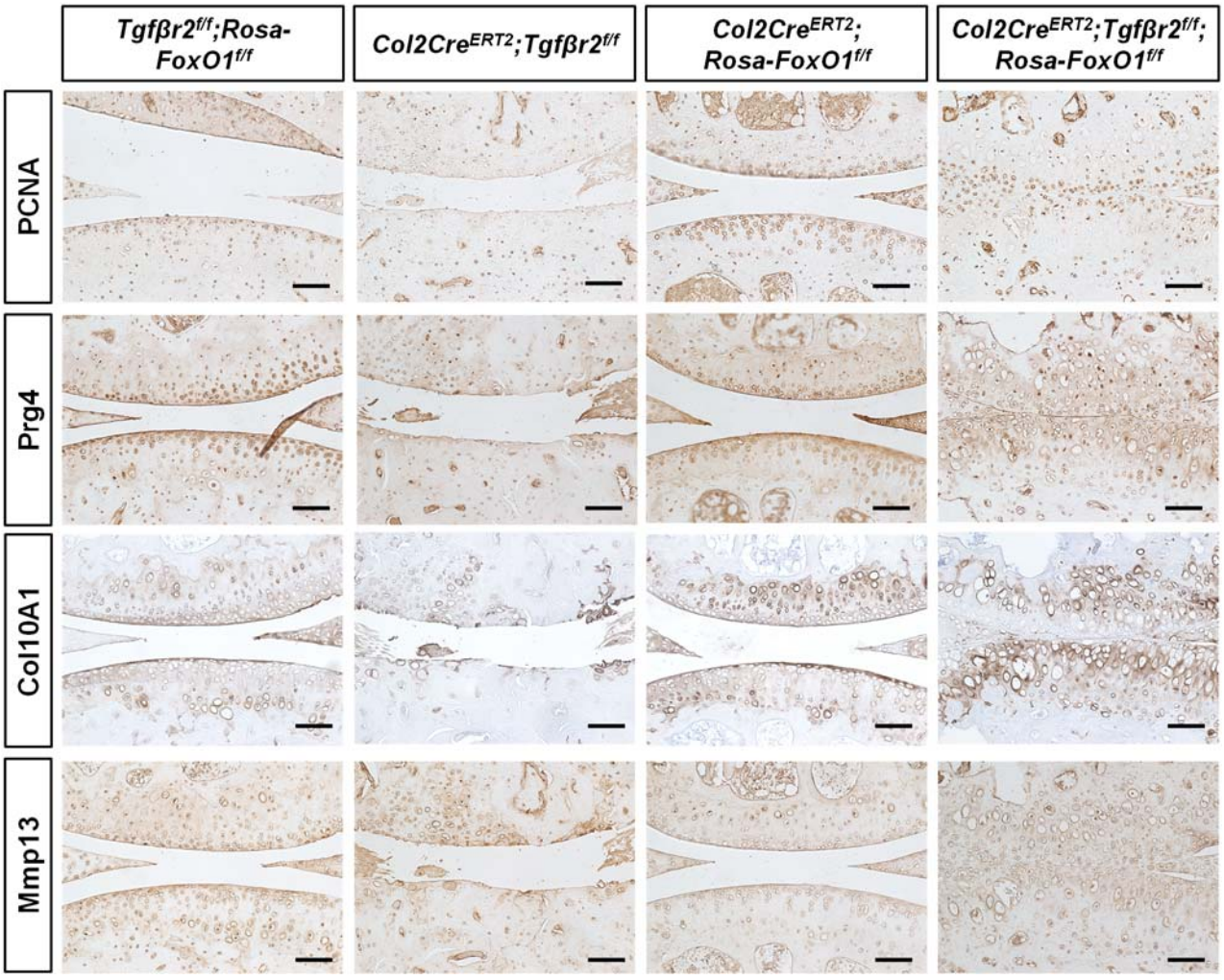

Fig. S13. FoxO1 GOF does not increase the autophagic flux in articular chondrocytes. Western blot analyses for protein expression in Ad-Con (Con) or Ad-Cre (Cre) transduced *Rosa-FoxO1$^{f/f}$* articular chondrocytes and treated with Bafilomycin (Baf) for the last 4 hours of the experiment. N = 3.

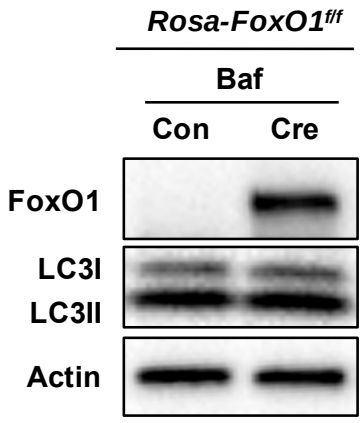

Table S1 Quantifications of trabeculae parameters of subchondral bone plate from 4- and 7-month-old *FoxO1*^{Agc1ER} and control mice. Data are means ± SD. N ≥ 5.

	4 mo		**7 mo**	
	***FoxO1*$^{f/f}$**	***Agc1Cre*^{ERT2}*; FoxO1*$^{f/f}$**	***FoxO1*$^{f/f}$**	***Agc1Cre*^{ERT2}*; FoxO1*$^{f/f}$**
BV/TV	0.743 ± 0.022	0.611 ± 0.057	0.722 ± 0.026	0.613 ± 0.040
Trabecular Thickness (mm)	0.092 ± 0.007	0.079 ± 0.008	0.095 ± 0.011	0.084 ± 0.006
Trabecular Spacing (mm)	0.044 ± 0.004	0.060 ± 0.010	0.050 ± 0.005	0.069 ± 0.011
Trabecular Number	23.127 ± 2.175	16.954 ± 2.624	20.153 ± 1.785	14.785 ± 2.415
Bone Mineral Density (mgHA/cm^3)	756.777 ± 21.914	631.797 ± 56.812	770.811 ± 33.415	652.615 ± 36.800

Table S2 Quantifications of trabeculae parameters of subchondral bone plate from control and *Rosa-FoxO1*^{Agc1ER} mice at 8 and 12 weeks following MLI injury. Data are means ± SD. N ≥ 5.

	8 wks following MLI		**12 wks following MLI**	
	***Rosa-FoxO1*$^{f/f}$**	***Agc1Cre*^{ERT2}*; Rosa-FoxO1*$^{f/f}$**	***Rosa-FoxO1*$^{f/f}$**	***Agc1Cre*^{ERT2}*; Rosa-FoxO1*$^{f/f}$**
BV/TV	0.784 ± 0.019	0.700 ± 0.064	0.735 ± 0.034	0.665 ± 0.038
Trabecular Thickness (mm)	0.113 ± 0.014	0.107 ± 0.020	0.111 ± 0.014	0.094 ± 0.006
Trabecular Spacing (mm)	0.040 ± 0.004	0.045 ± 0.006	0.054 ± 0.004	0.059 ± 0.010
Trabecular Number	24.993 ± 2.497	22.605 ± 2.906	18.504 ± 1.258	17.370 ± 3.537
Bone Mineral Density (mgHA/cm^3)	791.925 ± 28.076	712.828 ± 59.916	770.980 ± 40.955	697.589 ± 39.358

Table S3 Primer sequences for qPCR and ChIP-qPCR.

Genes	Sequences
qPCR-β-actin-F	5'-AGA TGT GGA TCA GCA AGC AG-3'
qPCR-β-actin-R	5'-GCG CAA GTT AGG TTT TGT CA-3'
qPCR-FoxO1-F	5’-AGT TCC TTC ATT CTG CAC TCG-3’
qPCR-FoxO1-R	5’-CTT CAA GGA TAA GGG CGA CAG-3’
qPCR-FoxO3-F	5’-CTG GGG GAA CCT GTC CTA TG-3’
qPCR-FoxO3-R	5’-TCA TTC TGA ACG CGC ATG AAG-3’
qPCR-FoxO4-F	5’-CTT CCT CGA CCA GAC CTC G-3’
qPCR-FoxO4-R	5’-ACA GGA TCG GTT CGG AGT GT-3’
qPCR-Agc1-F	5'-CGT GTT TCC AAG GAA AAG GA-3'
qPCR-Agc1-R	5'-TGT GCT GAT CAA AGT CCA G-3'
qPCR-Col2a1-F	5'-GCA GAG ATG GAG AAC CTG GTA-3'
qPCR-Col2a1-R	5'-AGC CTT CTC GTC ATA CCCT-3'
qPCR-Col10a1-F	5’-ATG CCT TGT TCT CCT CTT ACT G-3’
qPCR-Col10a1-R	5’-TGC TGA ACG GTA CCA AAC G-3’
qPCR-Mmp13-F	5'-AGA CTG GTA ATG GCA TCA AGG-3'
qPCR-Mmp13-R	5'-GCC ATT TCA TGC TTC CTG ATG-3'
ChIP-qPCR-Becn1-F	5'-TCG GCC CAC CTG TCG G 3'
ChIP-qPCR-Becn1-R	5'-CGA CGG GAG CTG ACG CAA GGA-3'
ChIP-qPCR-Map1lc3b-F	5’-CAT GCC TTG GGA CAC CAG AT-3’
ChIP-qPCR- Map1lc3b-R	5’-ACC TTC TTC AAG TGC TGT TTG T-3’