Supplemental information

Matrix IGF-1 regulates bone mass by activation of mTOR in Mesenchymal Stem Cells

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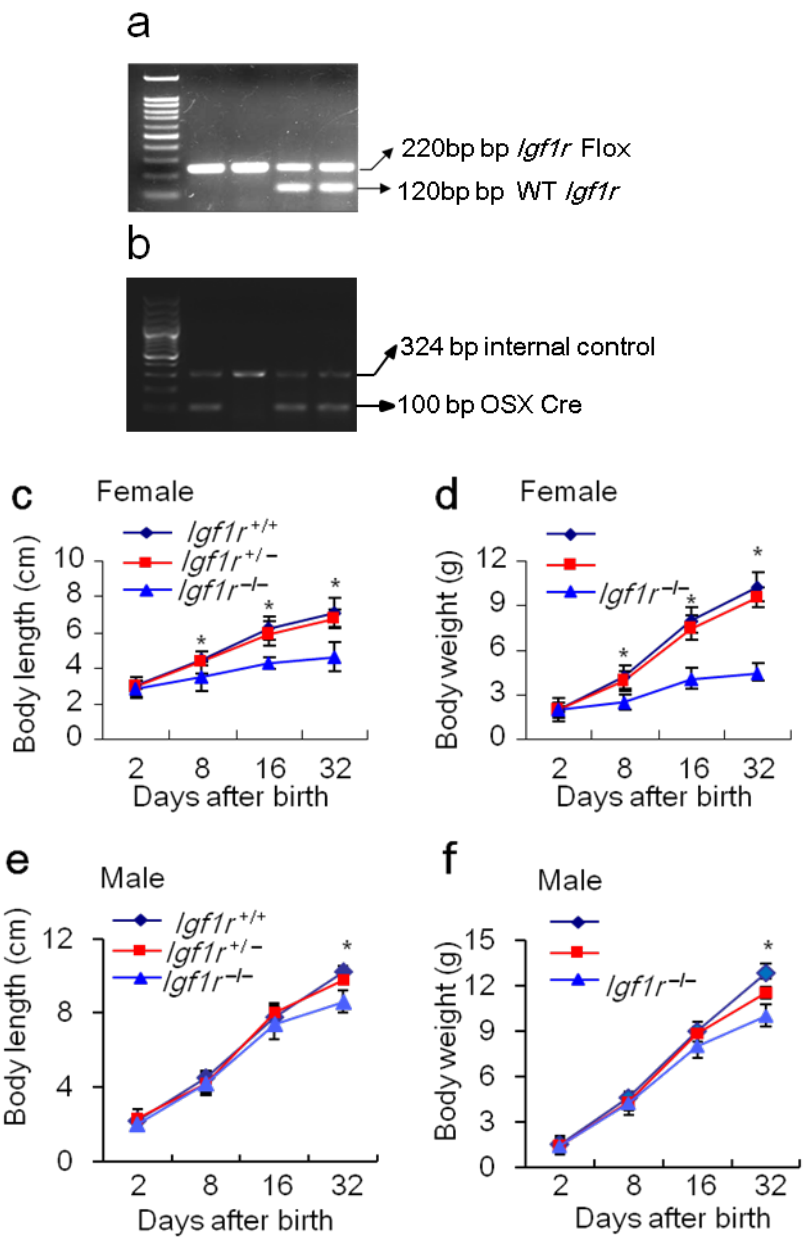
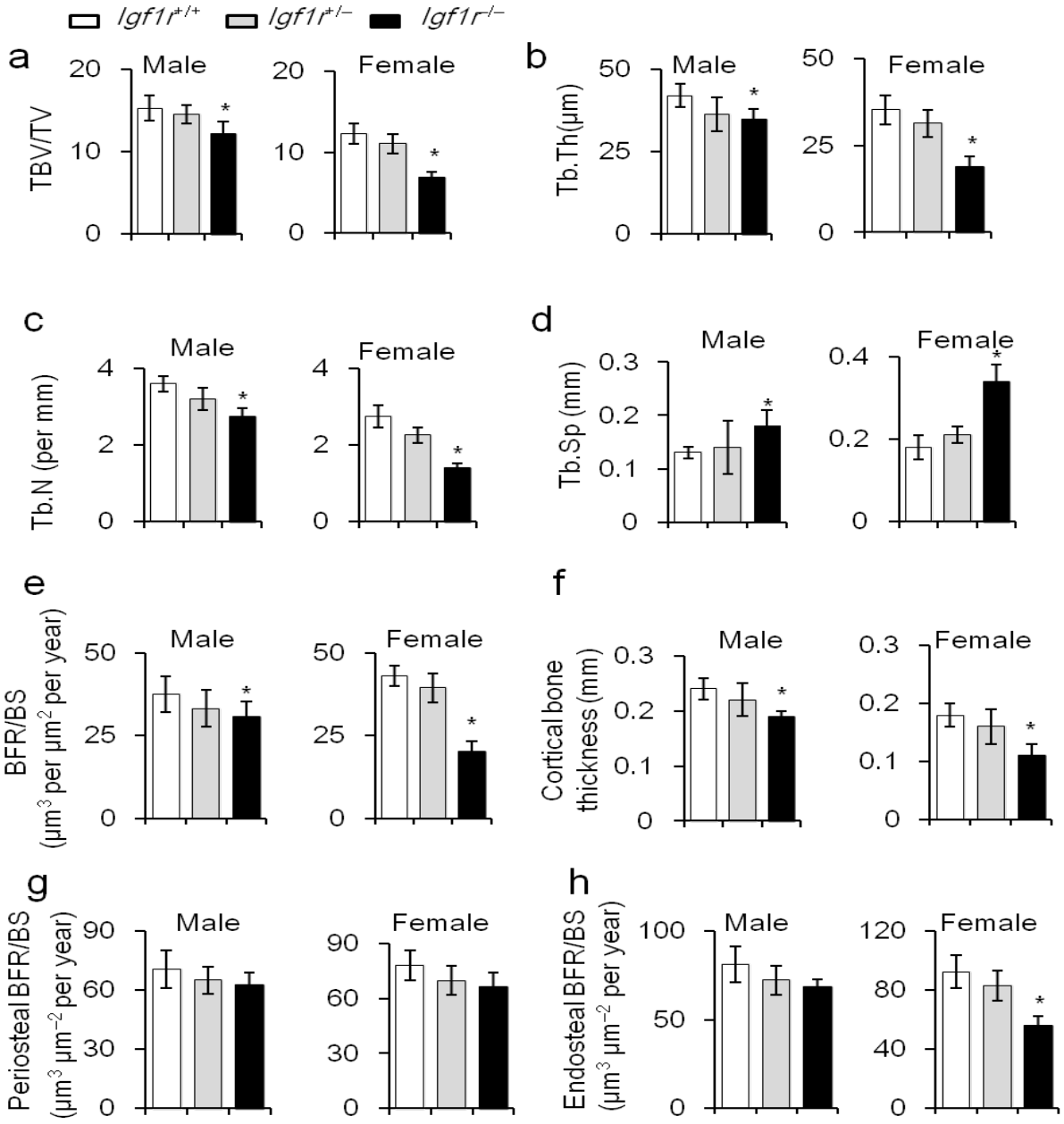


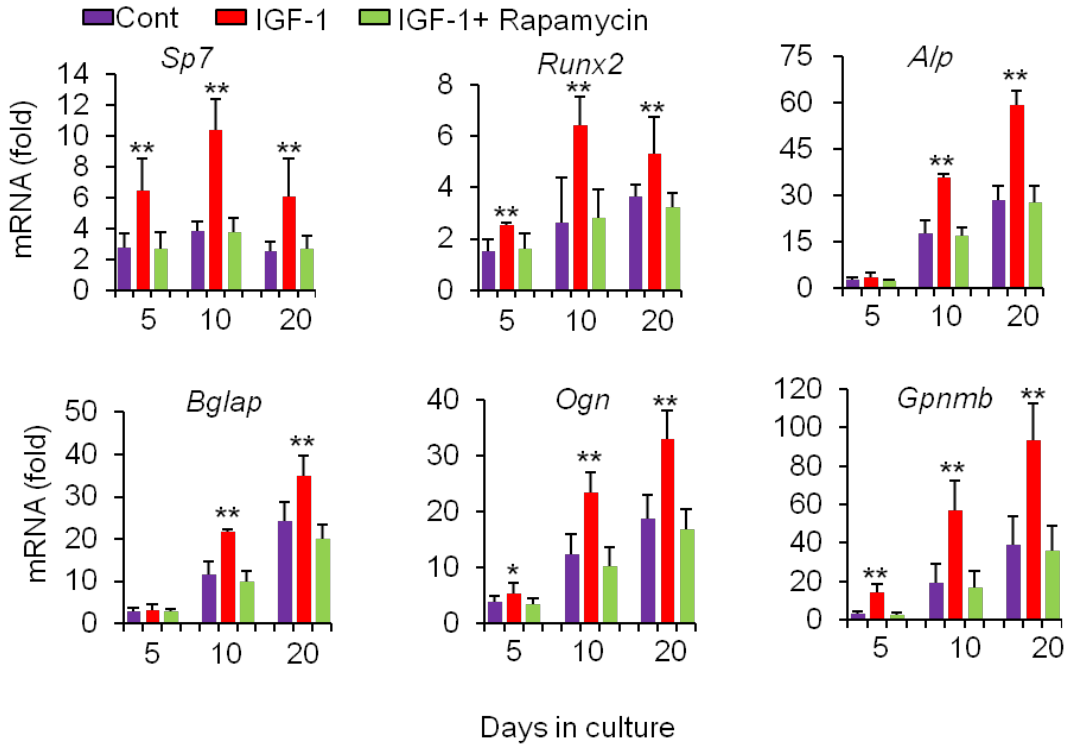
Supplementary Figure 1

Generation of *Igf1r*$^{-/-}$*(*Osx-Cre*; Igf1r*$^{fl/fl}$) and wild type *Igf1r*$^{+/+}$ (Osx-Cre*; Igf1r*$^{+/+}$) mice**. (**a**) Genotyping of the floxed and wild-type *Igf1r* alleles were conducted by PCR using specific primers, forward primer: 5'-CTTCCCAGCTTGCTACTCTAGG-3' and reverse primer: 5'-CAGGCTTGCAATGAGACATGGG-3'. The primers amplified the region spanning the downstream loxP sequence. (**b**) Genotyping of Cre transgenic mice was performed by PCR using primers detecting the Cre sequence. (**c**–**f**) Body length and body weight of male and female *Igf1r*$^{+/+}$, *Igf1r*$^{+/-}$ and *Igf1r*$^{-/-}$ mice were measured at different age points. Data represent the mean ± SEM (n = 10). *p < 0.05



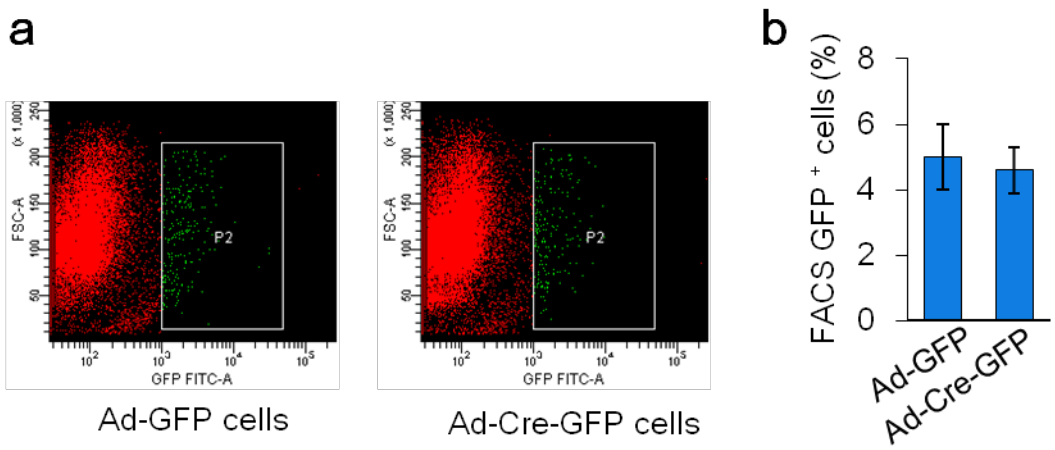
Supplementary Figure 2
Histomorphometrical analysis of *Igf1r*$^{-/-}$*(Osx-Cre; Igf1r*$^{fl/fl}$) mice in the secondary spongiosa of proximal tibiae and cortical bone of the tibial**

diaphysis. (**a**) Trabecular bone volume fraction (TBV/TV), (**b**) Trabecular Thickness(Tb.Th), (**c**) Trabecular number (Tb. N), (**d**) Trabecular Separation (Tb. Sp), (**e**) Bone formation rate per bone surface (BFR/BS), (**f**) Cortical bone thickness, (**g**) Periosteal bone formation rate per bone surface, (**h**) Endosteal bone formation rate per bone surface. Data represent the mean ± SEM. Female: *n* = 7-10. Male: 18-22. **p* < 0.05



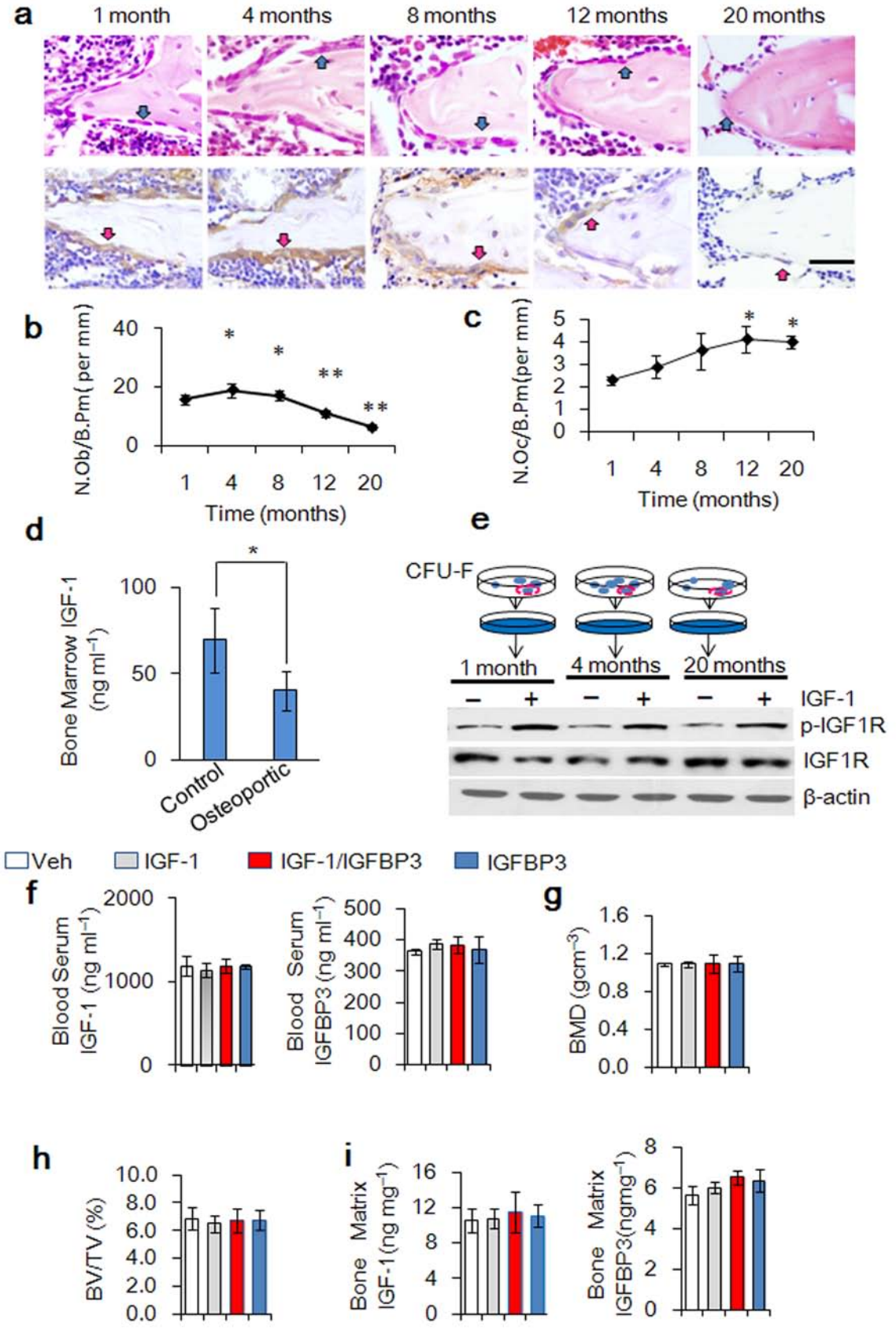
Supplementary Figure 3

Rapamycin inhibits expression of osteoblastic differentiation markers induced by IGF-1. MSCs were plated at 1,000 cells per well in 6-well plates in the presence or absence of IGF-1(100 ng ml^{-1}) with or without rampamycin (20 nM) in low serum differentiation medium. mRNAs of Osterix (*Sp7*), Runx2, Alkaline phosphatise (*Alp*), Osteocalcin (*Bglap*), Osteoglycin (*Ogn*), and Osteoactivin (*Gpnmb*) were assayed by real-time PCR at indicated time points. Data represent the mean ± SEM. *n* =5 **P<0.05*; ***P<0.01.*



Supplementary Figure 4

Survival rate of the transplanted GFP$^+$ Sca-1$^+$ MSCs infected with Ad-Cre-GFP or Ad-GFP in the bone marrow cavity. (**a**) GFP$^+$ cells from bone marrow and compact bones 2 weeks after transplantation were assessed by flow cytometry analysis. (**b**) Percentage of GFP$^+$ cells in bone marrow. Data represent the mean ± SEM, (*n* = 3).



Supplementary Figure 5

Age-related bone change assays from rats and its association with IGF-1 in the bone matrix (**a**) H&E staining (**top panels**) and immunohistology using antibody against Osteocalcin (**bottom panels**) of rat femur sections from mice at different ages as indicated. Arrows indicate osteoblasts. (**b, c**) Quantification of osteoblasts and osteoclasts in trabecular bone from mice with different ages. Number of osteoblast per bone perimeter (N.Ob/B.Pm); Number of osteoclast per bone perimeter (N.Oc/B.Pm); **(d)** Bone marrow IGF-1 concentration in osteoporotic individual (*n=12*) and comparative controls (*n=12*). Data represent mean ± SEM. *$p < 0.05$. (**e**) Effects of IGF-1 treatment (20 ng ml^{-1} for 10 minutes) on the levels of phosphorylated IGF1R *in vitro* cultured MSCs detected by western blot utilizing antibodies. Data represent the mean ± SEM. *$p < 0.05$. **(f)** IGF-1(**left**) and IGFBP3 (**right**) concentrations in blood serum from rats injected with vehicle, IGF-1, IGF-1 plus IGFBP3 or IGFBP3 only. Data represent the mean ± SEM. $n = 10$. (**g–i**) μCT analysis and measurement of the IGF-1 and IGFBP3 concentrations in bone matrix of the uninjected right femurs from the mice with injection of vehicle, IGF-1, IGF-1 plus IGFBP3 or IGFBP3 only in the left femurs. (**g, h**) Quantitative μCT analysis of the distal femora. Trabecular volumetric bone mineral density (TBMD) (**g**) and trabecular bone volume fraction (TBV/TV) (**h**)**.** (**i**) Concentrations of IGF-1 (**left**) and IGFBP3 (**right**) in bone matrix extraction. Data represent the mean ± SEM. $n = 10$. *$p < 0.05$.

Supplementary Table 1 μCT analysis of distal femurs from mice with rapamycin injection

	Veh	Rapamycin
Trabecular bone volume BV/TV (%)	9.5±0.42	6.94±0.54**
Bone mineralized density BMD (g cm^{-3})	1.30±0.07	1.12±0.03**
Mean trabecular pattern factor Tb.pf (per mm)	9.2±0.88	7.70±1.9*
Trabecular separation Tb.Sp (mm)	0.2±0.06	0.32±0.078**
Trabecular thickness Tb.Th (mm)	0.017±0.008	0.012±0.002*
Trabecular number Tb.N (per mm)	6.7±1.2	4.2±0.63*

Data represent the mean ± SEM. $n = 8$. *$p < 0.05$, ** $p < 0.01$

Supplementary Table 2 Clinical characteristics of human samples

	Control	Osteoportic
Age (years)	72.1±6.9	72.8±9.4
Weight (kg)	67.7±11.9	56.3±10.8**
Height (cm)	153.7±6.8	150.9±6.4
BMD (g cm^{-2})	1.19±0.2	0.8±0.1**
t-score (L2-L4)	-0.68±1.4	-3.6±0.8**
z-score (L2-L4)	1.03±1.3	-1.5±0.7**
BMI (kg m^{-2})	28.1±4.7	25.4±3.4*
Fat (%)	42.0±7.8	32.3±15.2*
Bone marrow IGF-1(ng ml^{-1})	69.8±18.7	40.3±11.1*

Data represent the mean ± SEM. n = 12. *p < 0.05, ** p < 0.01.

Supplementary Table 3 µCT Analysis of the distal femurs from vehicle, IGF-1, or IGF-1 plus IGFBP3-infused LID mice

		LID		
	WT	**Veh**	**IGF-1**	**IGF-1/IGFBP3**
Trabecular bone volume BV/TV (%)	7.62±0.29	6.98±0.18#	7.01±0.24#	8.36±0.25#*
Bone mineralized density BMD (g cm^{-3})	1.31±0.04	1.2±0.02#	1.19±0.05#	1.34±0.04*
Trabecular number Tb.N (per mm)	3.67±0.40	3.37±-0.11	3.16±0.17	3.79±0.14*
Trabecular thickness Tb.Th (mm)	0.019±0.0002	0.016±0.0008#	0.015±0.0006#	0.019±0.007*
Mean trabecular pattern factor Tb.pf (per mm)	15.29±3.26	16.64±3.36	16.71±2.08	15.61±1.21
Trabecular separation Tb.Sp (mm)	0.187±0.014	0.194±0.009	0.192±0.007	0.176±0.013*

Data represent the mean ± SEM. n = 6. #p < 0.05 *vs* WT, * p < 0.01 *vs* Veh.