		12 weeks		28 weeks	
		$ER\alpha^{f/f}$ (N=10)	$ER\alpha^{f/f}$;Prx1-cre (N=10)	$ER\alpha^{f/f}$ (N=4)	$ER\alpha^{f/f}$;Prx1-cre (N=5)
Distal	BV/TV (%)	10.4±1.9	9.4±2.3	2.6±1.2	3.8±1.3
	BMD (mg/cm^3)	119.4±17.3	110.4±17.9	24.6±9.8	28.6±14.1
	Tb Thickness (μm)	53.4±3.4	54.4±3.8	46.5±10.0	56.5±2.7
	Tb Number (/mm)	3.8±0.3	2.9±0.4 *	2.5±0.2	2.0±0.4
	Tb Spacing (μm)	265.6±18.2	353.9±50.0 *	396.6±27.8	511.1±112.2
Midshaft	BMD (mg/cm^3)	1184.1±57.5	1144.9±24.3	1388.2±12.8	1322.0±24.8 *
	Cortical Thickness (mm)	0.17±0.013	0.16±0.008 *	0.22±0.007	0.19±0.011 *
	Femoral length (mm)	14.2±0.4	14.0±0.7	15.9±0.3	15.9±0.9
	Uterine Weight (mg)	46.9±9.7	47.1±2.0	35.0±10.2	39.6±6.5
	Body Weight (g)	18.1±1.1	19.3±1.6	20.8±1.6	20.6±1.8

Supplemental Table 1

Deletion of ERα in Prx1-cre expressing cells decreases cortical bone mass. Femur micro-CT, length, and weight measurements in female mice. BV/TV=bone volume per tissue volume, BMD=bone mineral density, Tb=Trabecular. Data represent mean and s.d.; *$p<0.05$ versus respective $ER\alpha^{f/f}$ littermates by Student's t-test.

		12 weeks		26 weeks	
		$ER\alpha^{f/f}$ (N=11)	$ER\alpha^{f/f}$;Col1a1-cre (N=14)	$ER\alpha^{f/f}$ (N=8)	$ER\alpha^{f/f}$;Col1a1-cre (N=11)
Vertebra	BMD (mg/cm^3)	282±30	310±52	262±33	276±34
	Tb Thickness (μm)	79.1±8.0	86.6±7.4 *	72.1±4.9	73.6±3.8
	Tb Number (/mm)	4.8±0.4	4.9±0.6	5.4±0.4	5.6±0.5
	Tb Spacing (μm)	215±27	210±2.2	265±19	252±32
Femur	BMD (mg/cm^3)	147±23	159±50	52.7±15.4	55.4±21.0
	Tb Thickness (μm)	53.4±3.1	54.3±9.1	58.5±6.4	55.8±8.3
	Tb Number (/mm)	4.1±0.8	4.0±0.8	2.5±0.4	2.6±0.3
	Tb Spacing (μm)	253±59	263±5.2	408±78	382±43
	Body Weight (g)	23.3±1.9	22.8±2.2	32.0±4.5	30.9±4.9

Supplemental Table 2

Cancellous bone mass is unaffected by deletion of ERα in Col1a1-cre expressing cells. Micro-CT measurements in vertebra and distal femur of female mice. BMD=bone mineral density, Tb=Trabecular. Data represent mean and s.d.; *$p<0.05$ versus respective $ER\alpha^{f/f}$ littermates by Student's t-test.

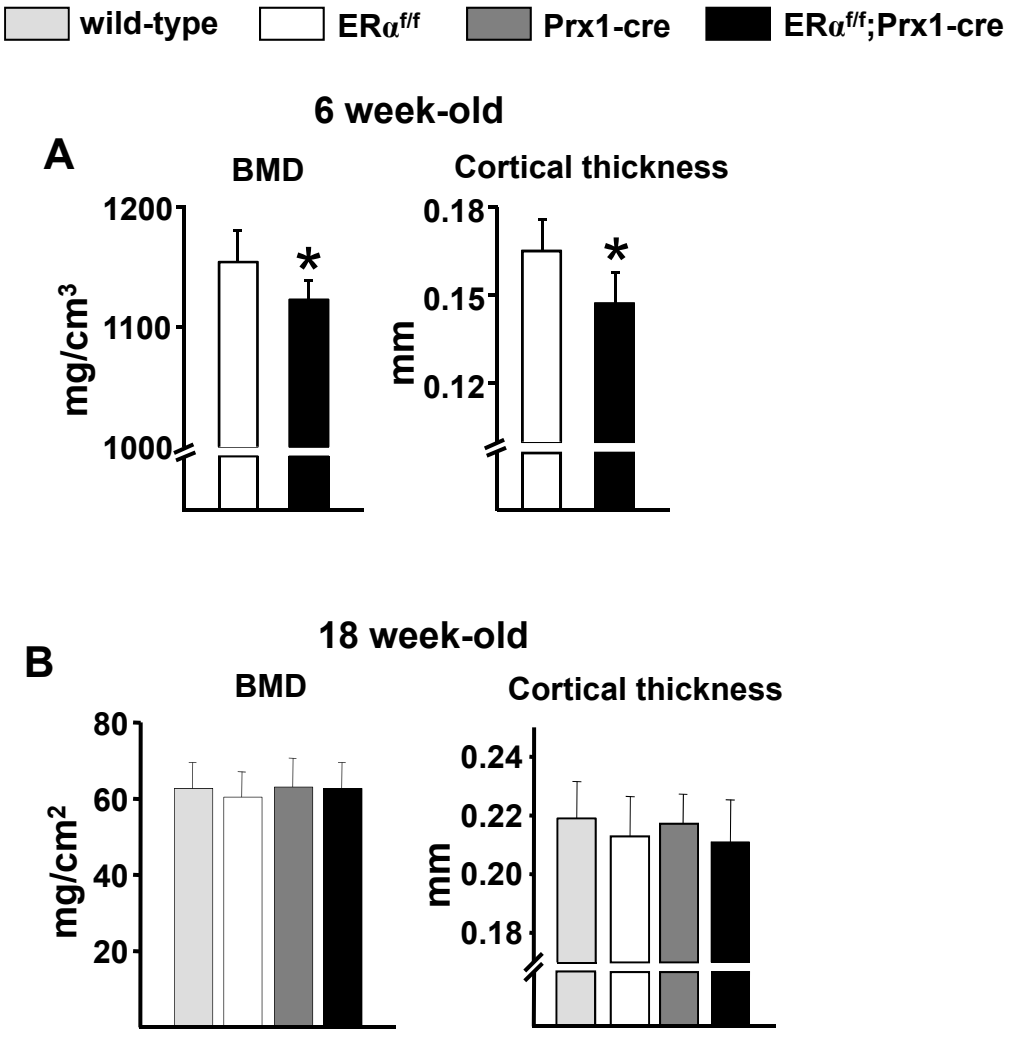

Figure S1

Femoral bone mass in ERα$^{f/f}$;Prx1-cre male mice. (**A**) BMD and cortical thickness determined by micro-CT at the femoral midshaft (n=5-7/group). (**B**) BMD of the whole femur determined by DEXA and cortical thickness determined by micro-CT at the femoral midshaft (n=7-12/group). Bars represent mean and s.d.; *p<0.05 by Student's t-test.

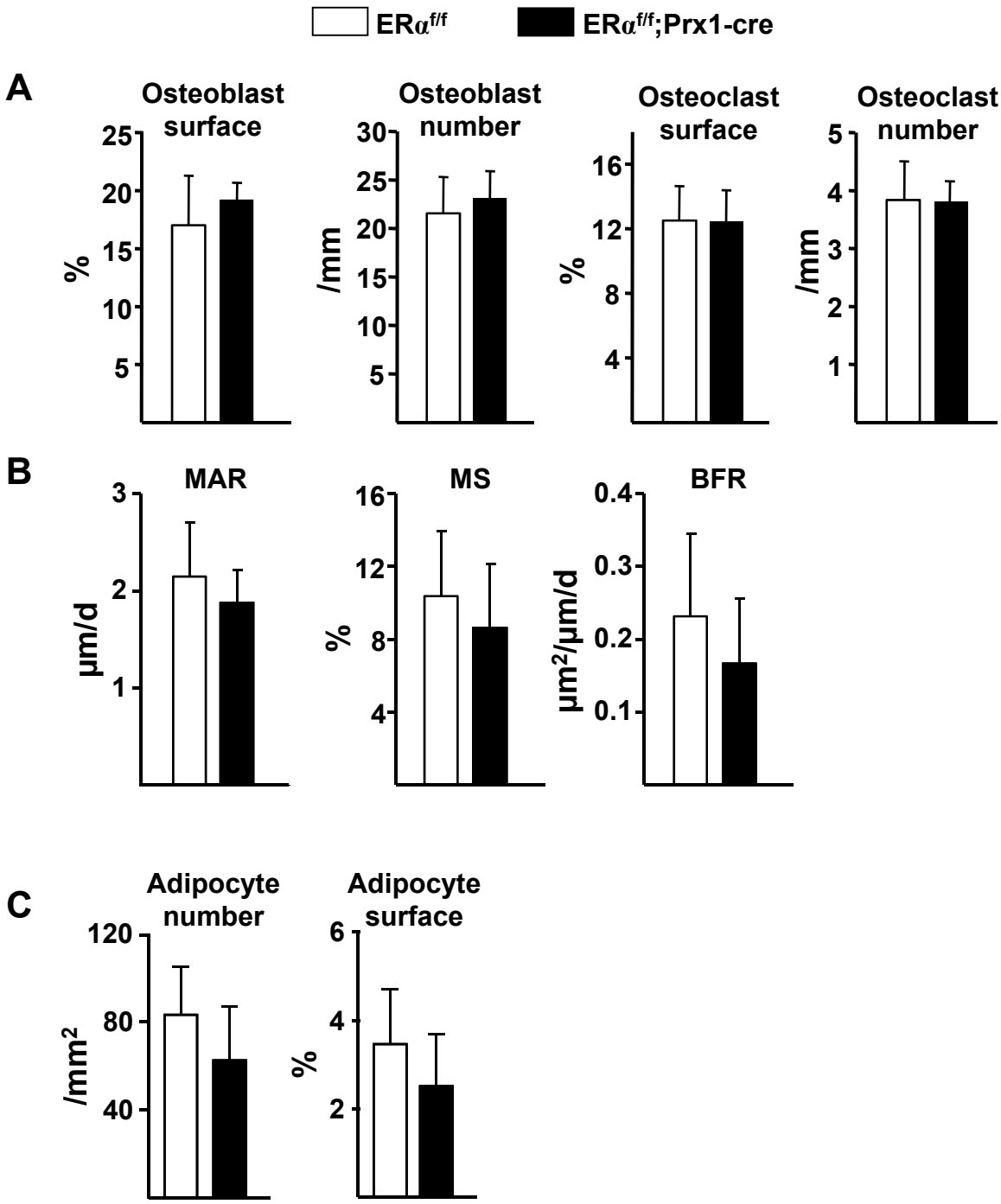

Figure S2

Deletion of ERα in Prx1-cre expressing cells does not affect cancellous bone and marrow adipogenesis. (**A**) Osteoblast and osteoclast number and surface per mm of bone surface; and (**B**) mineral apposition rate (MAR), mineralizing surface (MS), and bone formation rate (BFR) as determined by tetracycline labels in the cancellous bone of the distal end of femurs determined by histology of longitudinal undecalcified sections from 8 week old female (n=6-9/group). (**C**) Adipocyte number and area per tissue area in the bone marrow of the distal end of femurs determined by histology of longitudinal decalcified sections from 12 week old female mice (n=9-10/group).
Bars represent mean and s.d.

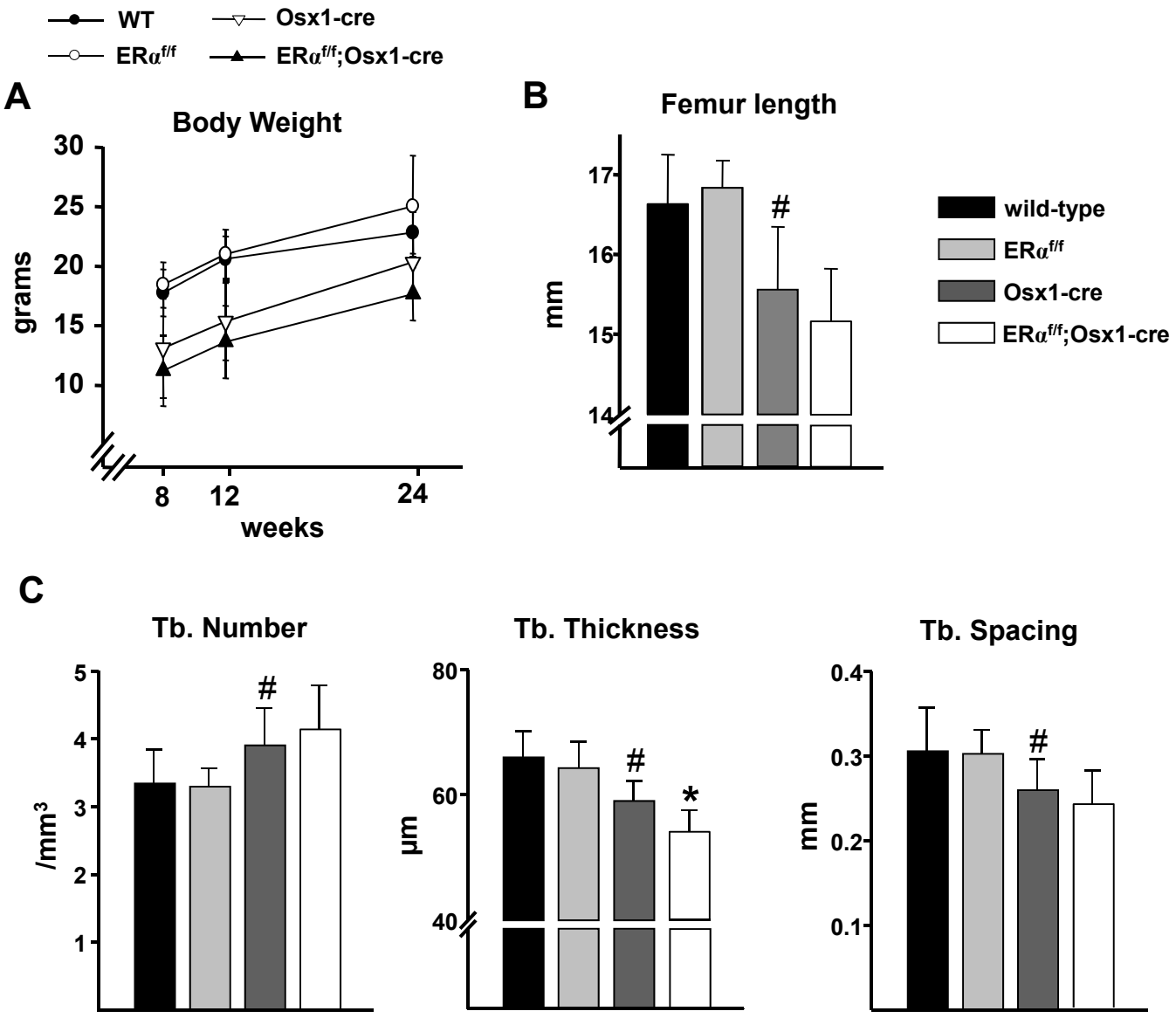

Figure S3

Femoral length and cancellous bone mass are unaffected by deletion of ERα in Osx1-Cre expressing cells. (**A**) Longitudinal measurements of body weight, (**B**) femur length and (**C**) micro-CT measurements in the 5th lumbar vertebra of 24-week-old female mice (n=6-11/group). BV/TV= bone volume per tissue volume, Tb=Trabecular. Bars represent mean and s.d.; *p<0.05 versus Osx-cre, # p<0.05 versus wild-type or ERα$^{f/f}$ by two-way ANOVA.

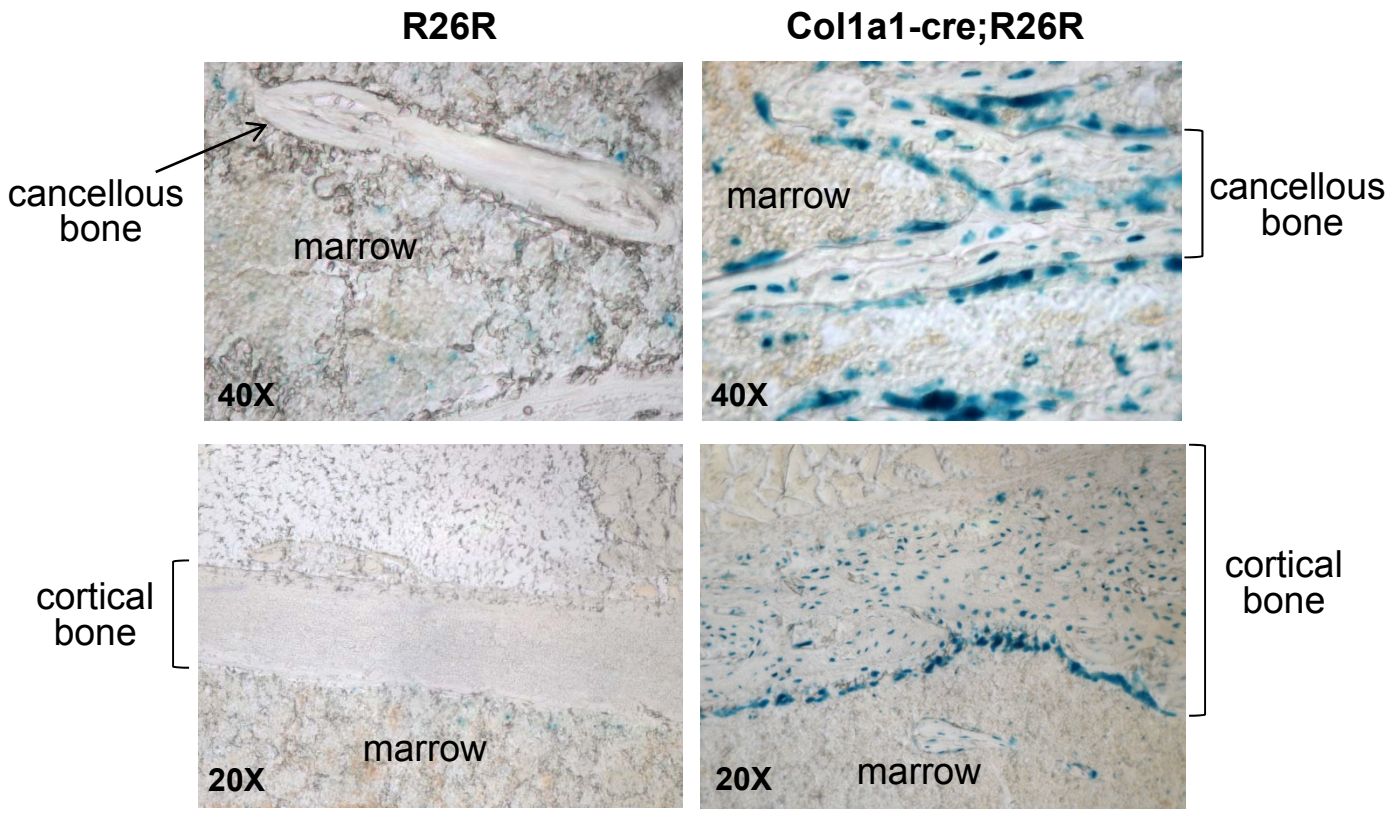

Figure S4

Efficiency of Col1a1-cre-mediated recombination in osteoblasts and osteocytes. X-gal stained histological frozen sections of the distal femur of 5 week old R26R control and Col1a1-cre;R26R mice.

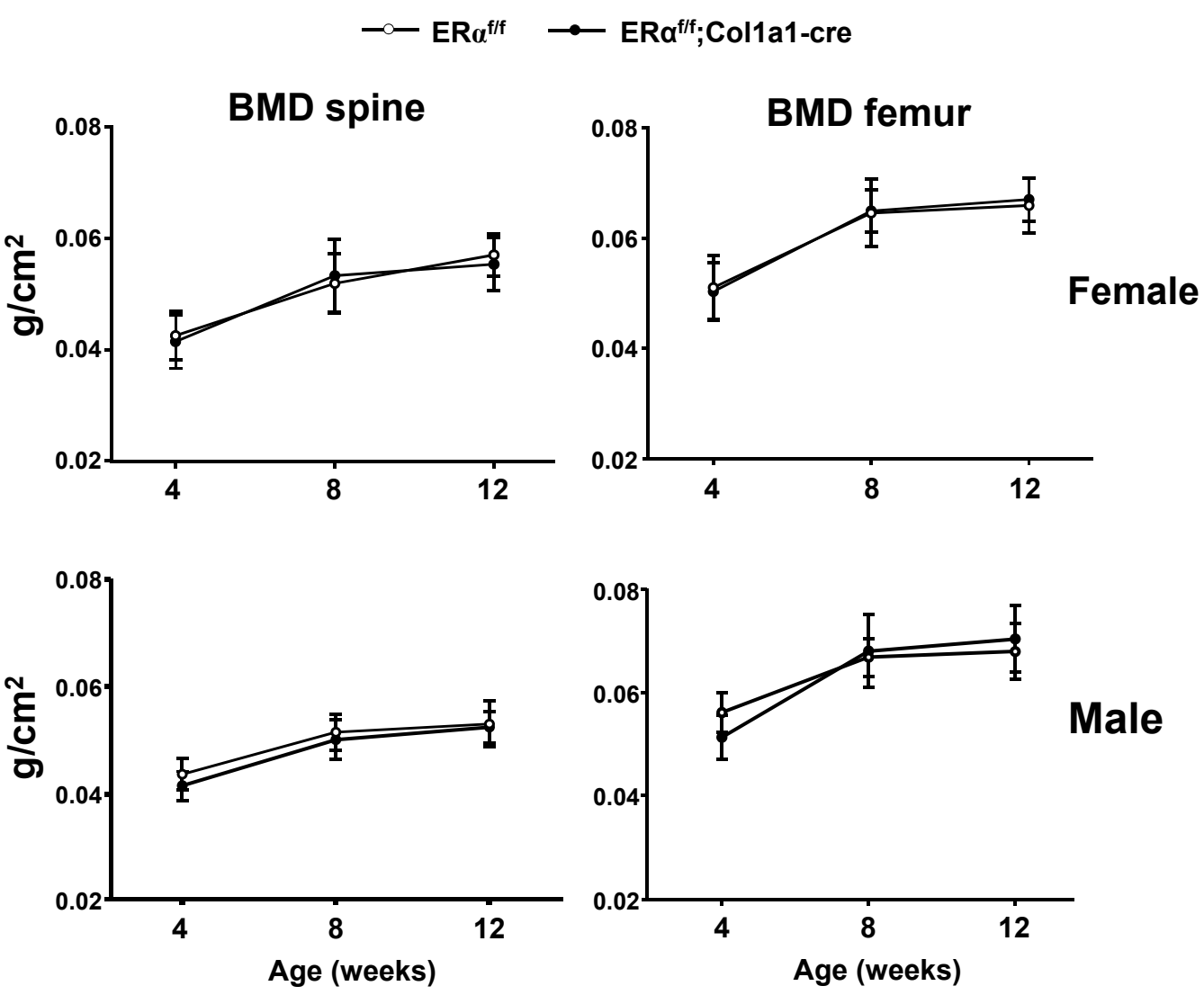

Figure S5

Bone mass is unaffected in ERα$^{f/f}$;Col1a1-cre mice. Longitudinal BMD measurements by DEXA in female (n=11-14/group) and male (n=11-12/group) mice. Data represent mean and s.d.

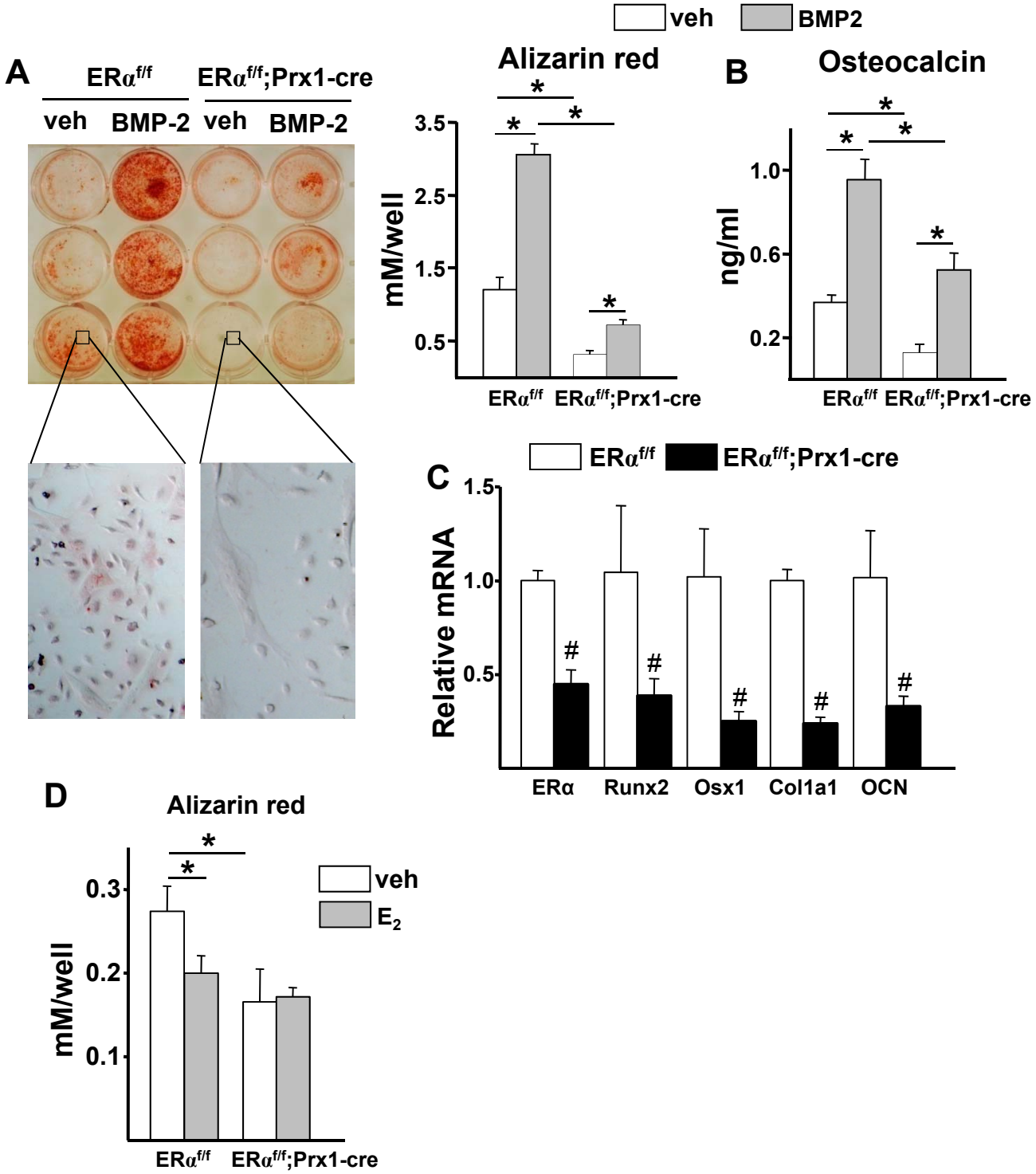

Figure S6

Osteoblastogenesis is attenuated in bone marrow-derived osteoblastic cells from ERα$^{f/f}$;Prx1-cre mice. (**A**) Mineralized matrix visualized and quantified following Alizarin red staining and **(B)** osteocalcin levels in the medium of cell cultures pooled from 3 mice treated with vehicle (veh) or rhBMP2 (25 ng/ml) for 18 days (triplicate cultures). (**C**) mRNA levels of the indicated genes determined by quantitative PCR in cells cultured with ascorbic acid for 14 days. (**D**) Mineralized matrix quantified following Alizarin red staining in cells cultured with veh or E_2 (10^{-8} M) in the presence of ascorbic acid for 21 days (triplicate cultures). Bars represent mean and s.d.; *p<0.05 by two-way ANOVA, # p<0.05 by Student's t-test.

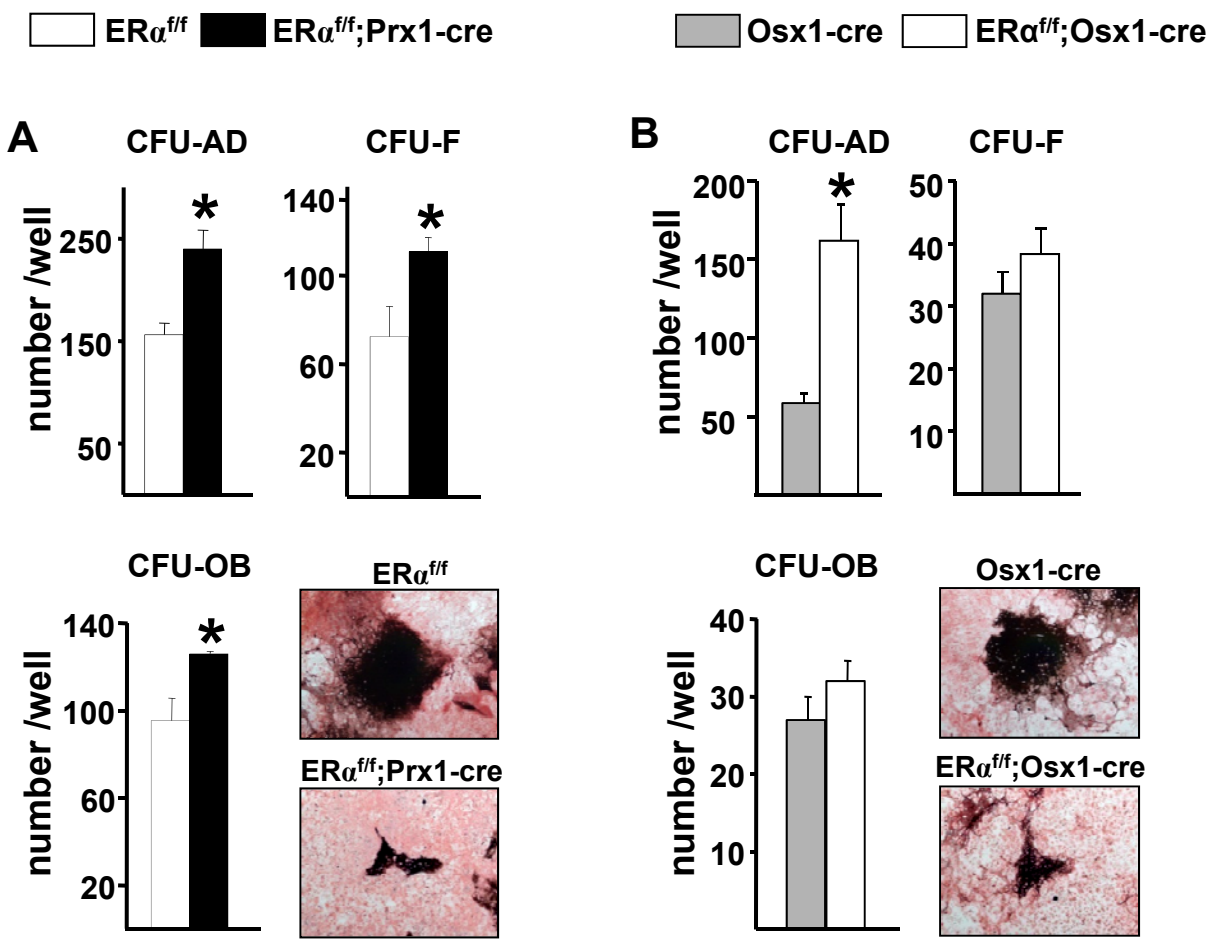

Figure S7

Mesenchymal progenitors are increased in ERα$^{f/f}$;Prx1-cre. (**A-B**) Bone marrow cells from the femurs of 3 mice were pooled and plated in triplicate at: 1x10^6 cells/well for CFU-Ad, 1x10^6 cells/well for CFU-F, or 2x10^6 cells/well for CFU-Ob. CFU-Ad were stained with Oil Red O after 7 days, CFU-F stained for alkaline phosphatase after 10 days, and CFU-Ob stained with von Kossa after 25 days to detect mineral. Photomicrographs show representative CFU-Ob colonies (50x). Bars represent mean and s.d.; *p<0.05 by Student's t-test.

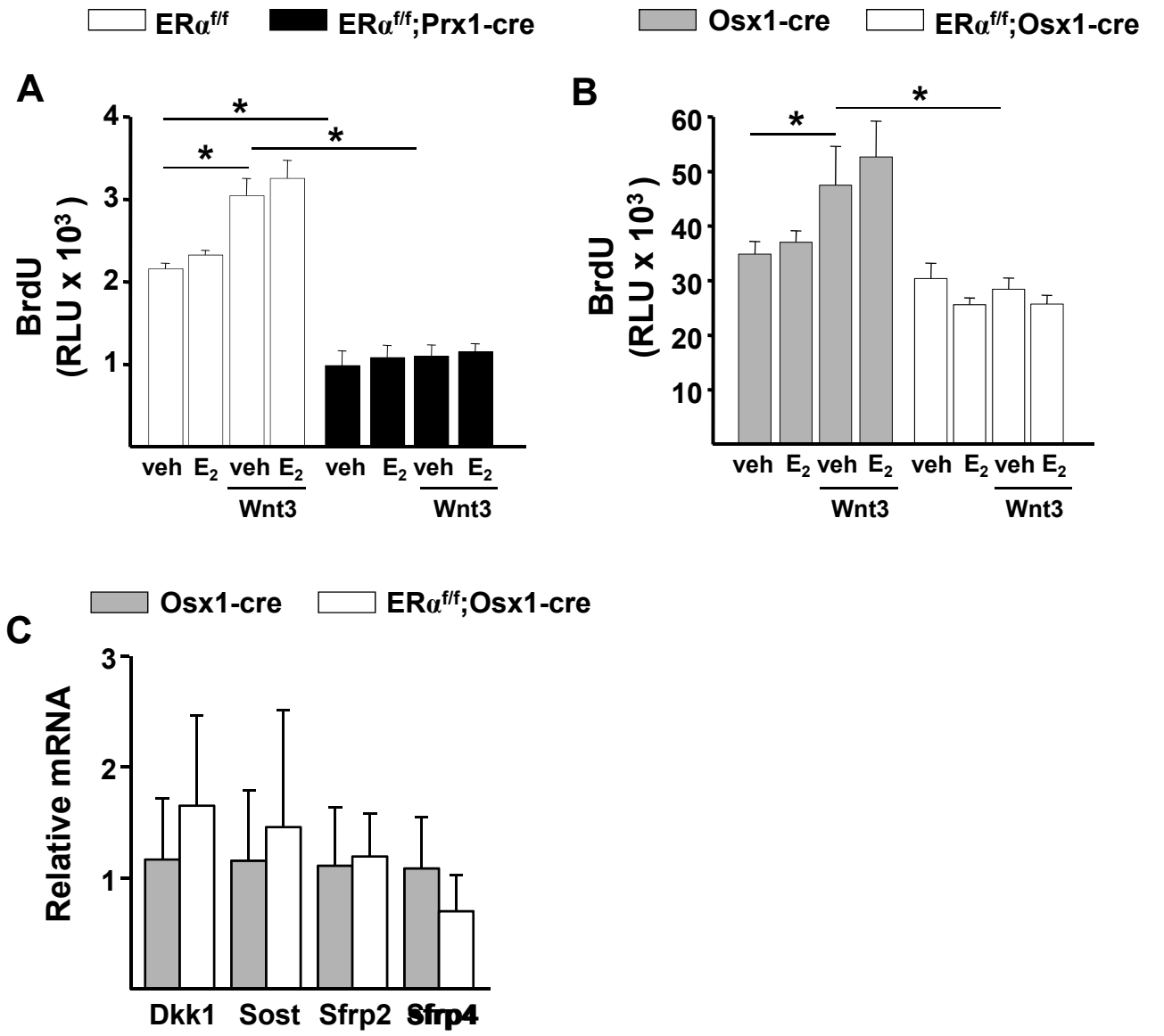

Figure S8

The unliganded ERα potentiates Wnt-induced cell proliferation. BrdU incorporation in bone marrow-derived osteoblastic cell cultures pooled from 3 mice of each: (**A**) ERα$^{f/f}$;Prx1-cre and ERα$^{f/f}$ littermates and (**B**) ERα$^{f/f}$;Osx1-cre and Osx1-cre littermates, pre-incubated for 1 h with vehicle (veh) or E_2 (10^{-8} M) followed by incubation without or with Wnt3 (25 ng/ml) for 3 days. (**C**) mRNA levels of the indicated genes determined by quantitative PCR in calvaria bone from 24 week old mice (n=7-9/group). Bars represent mean and s.d.; *p<0.05 by one-way ANOVA with Bonferroni's test.

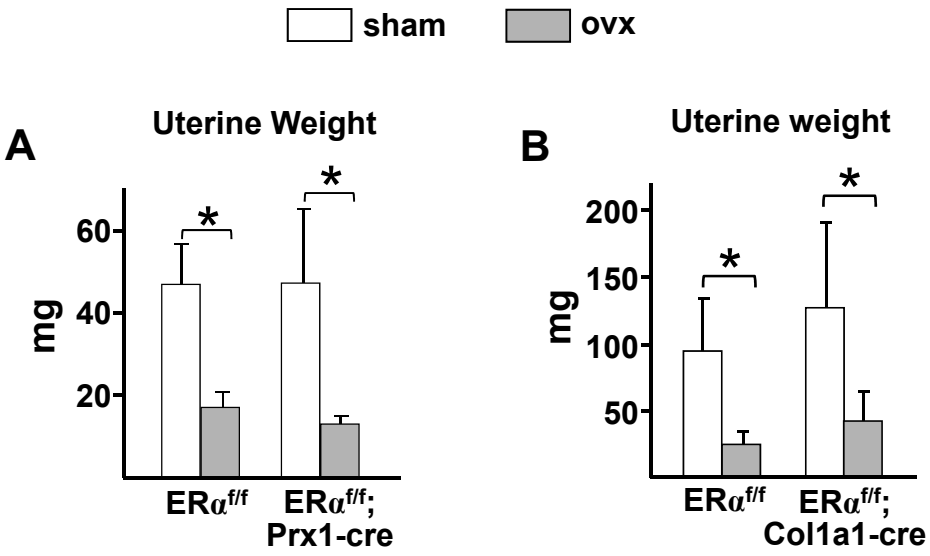

Figure S9

Uterine weight in ovariectomized ERα$^{f/f}$;Prx1-cre and ERα$^{f/f}$;Col1-cre mice. (**a**) Eight week-old mice were sham-operated or ovariectomized and euthanized 3 weeks later (n=10/group). (**b**) Twenty week-old mice were sham-operated or ovariectomized and euthanized 6 weeks later (n=11/group). Bars represent mean and s.d.; * $p<0.05$ by two-way ANOVA.

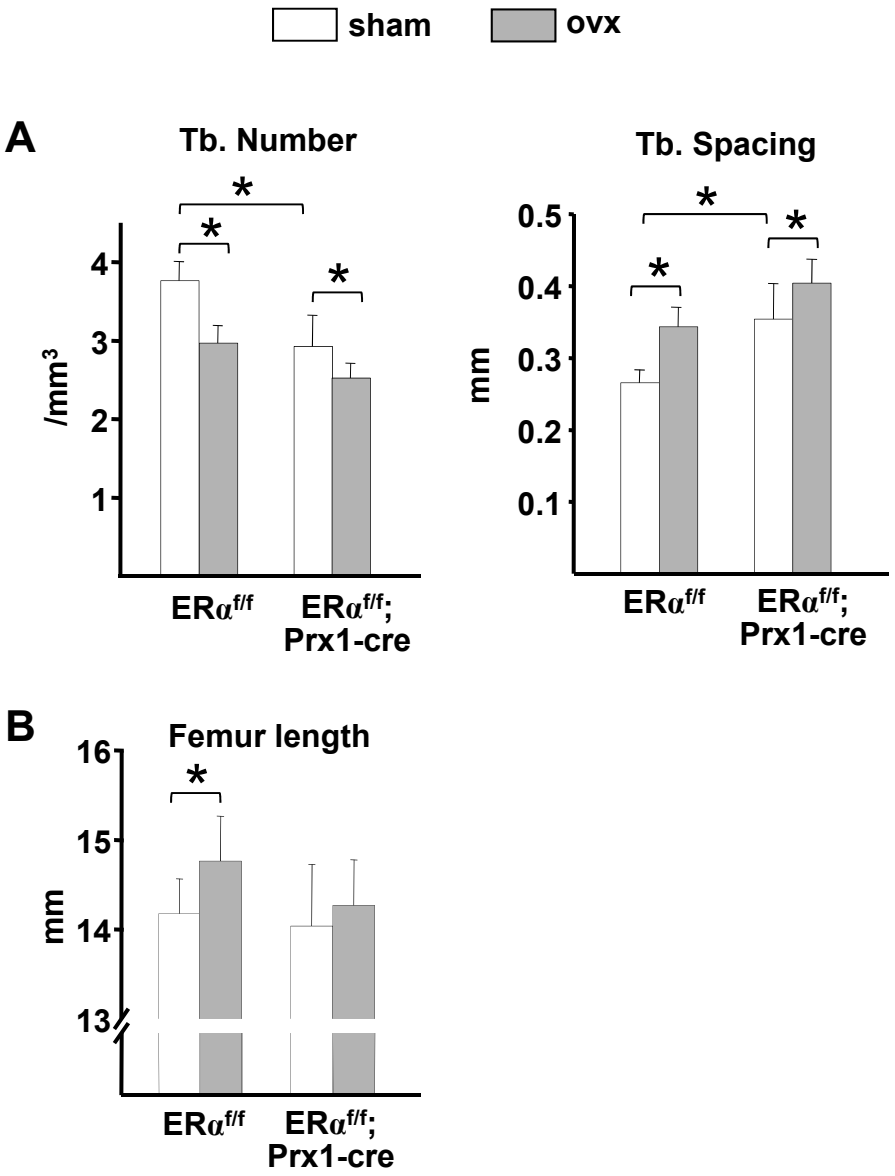

Figure S10

Cancellous bone microarchitecture and femur length in ovariectomized ERα$^{f/f}$;Prx1-cre mice. Eight week-old female mice were sham-operated or ovariectomized and euthanized 3 weeks later. (**A**) Micro-CT measurements were performed at the distal femur (n=10/group). (**B**) Femur length was measured with calipers. Bars represent mean and s.d.; * $p<0.05$ by two-way ANOVA.