Supplemental data

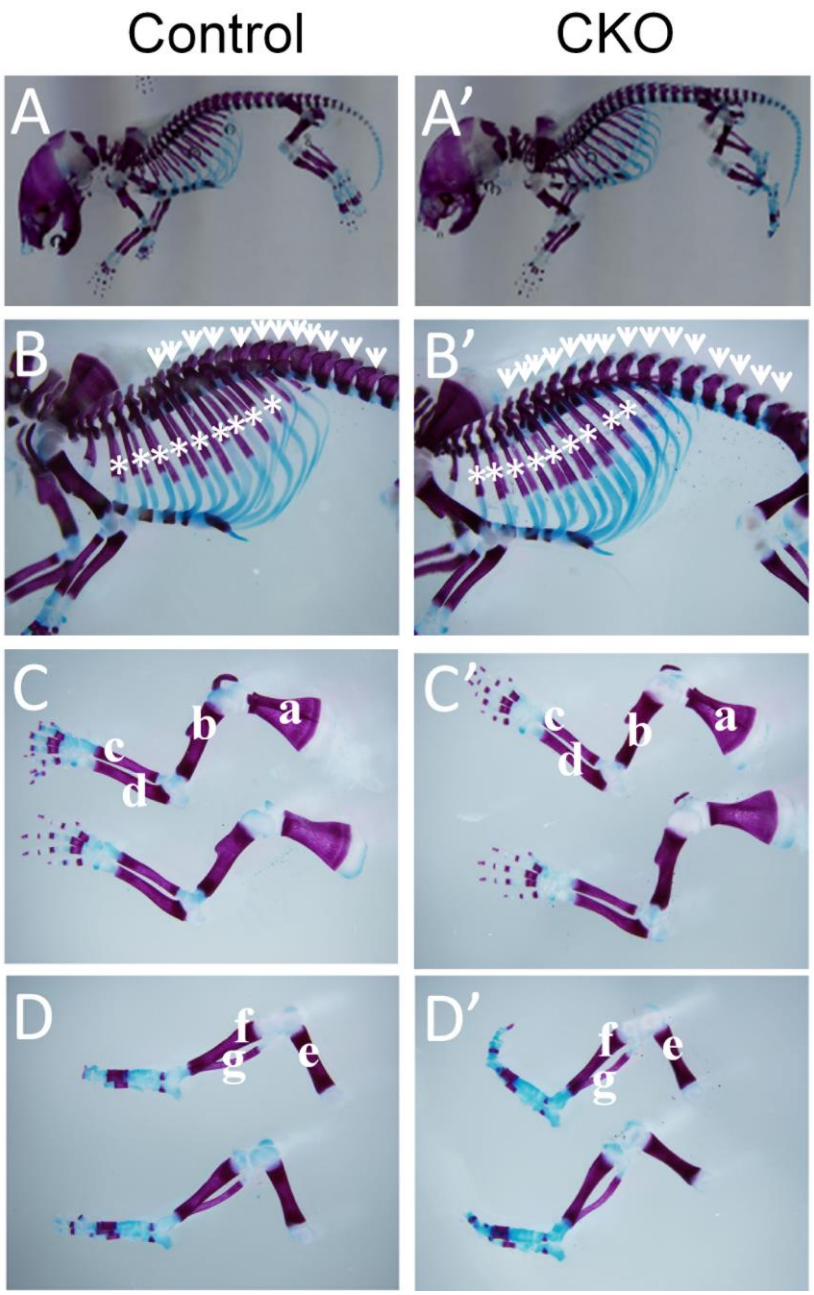

Figure S1. TSC1 deletion by Osx-Cre does not affect embryonic skeleton development. Skeleton preparation of *Tsc1*$^{F/F}$ (Control) (A-D) and *Tsc1*$^{F/F}$;Osx-Cre (CKO) (A'-D') mice at birth. (A, A') Overall staining. (B, B') Thoracic cage (arrow heads point to vertebrae and * indicates rib). (C, C') Arm (a: scapula, b: humerus c: radius d: ulna). (D, D') Leg (e: femur, f: tibia, g: fibula).

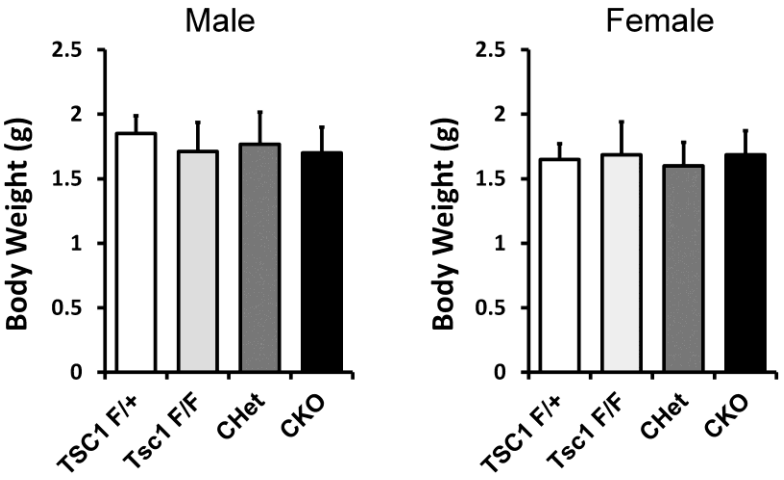

Figure S2. *Tsc1* deletion by Osx-Cre did not affect body weight in neonatal mice. Body weight of *Tsc1*$^{F/+}$, *Tsc1*$^{F/F}$, *Tsc1*$^{F/+}$;Osx-Cre (CHet) and *Tsc1*$^{F/F}$;Osx-Cre (CKO) mice at one-day old. n=6 for *Tsc1*$^{F/+}$, n=8 for *Tsc1*$^{F/F}$, n=6 for CHet, and n=8 for CKO male mice. n=6 for *Tsc1*$^{F/+}$, n=7 for *Tsc1*$^{F/F}$, n=4 for CHet, and n=7 for CKO female mice. Data were presented as mean + SE.

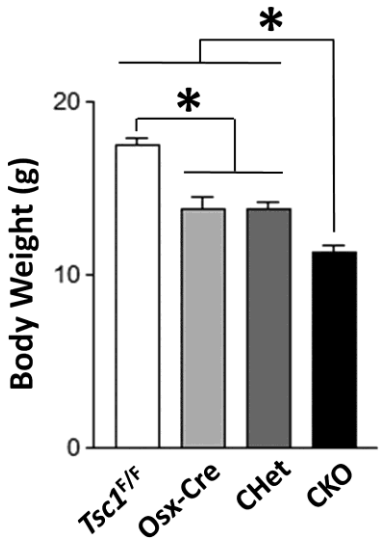

Figure S3. *Tsc1* deletion by Osx-Cre leads to decreased body weight at one month old. Body weight of *Tsc1*$^{F/F}$, Osx-Cre, *Tsc1*$^{F/+}$;Osx-Cre (CHet) and *Tsc1*$^{F/F}$;Osx-Cre (CKO) male mice at one month old. n=41 for *Tsc1*$^{F/F}$, n=13 for Osx-Cre, n=43 for CHet, and n=45 for CKO mice. Data were presented as mean + SE. * $p<0.05$

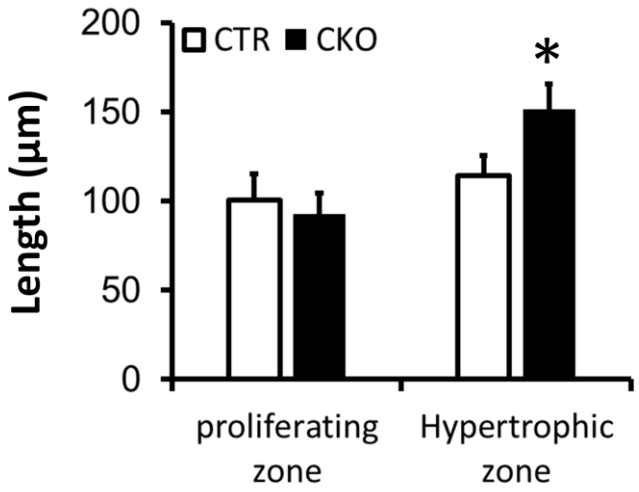

Figure S4. *Tsc1* deletion by Osx-Cre leads to increased length of hypertrophic zone in femoral growth plate. *p<0.05, n=5. Values were presented as mean + SE.

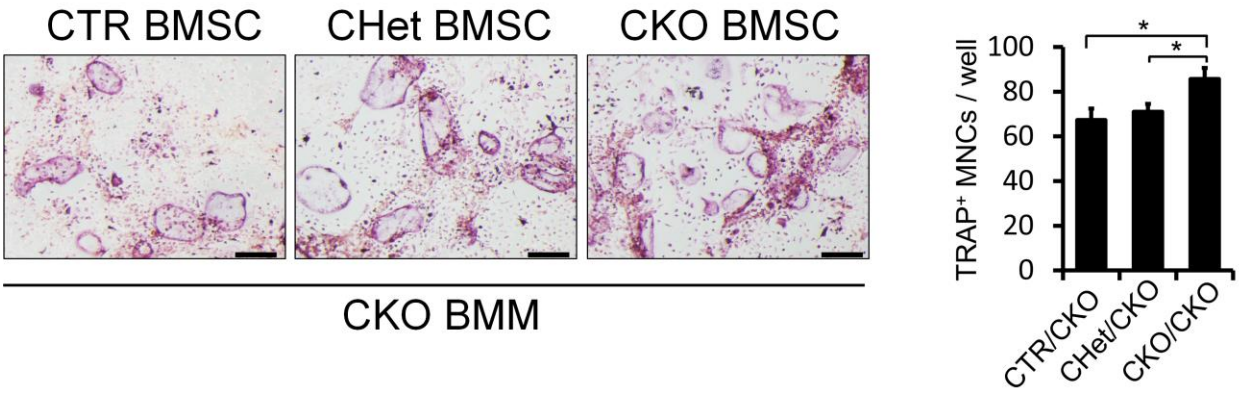

Figure S5. TSC1-deficient BMSCs supports osteoclastogenesis

50,000 Bone marrow derived macrophages (BMMs) isolated from CKO mice were cultured with 10,000 BMSCs isolated from CTR, CHet and CKO mice in the presence of 20 nM 1α, 25-dyhydroxy vitamin D_3 (Sigma) and 1 μM prostaglandin E2 for 7 days. Numbers of TRAP-positive ($TRAP^+$) multinucleated cells (MNC) (>3 nuclei) were counted (right panel). Scale bar=100μm. *p<0.05, n=3. Values were presented as mean + SE.

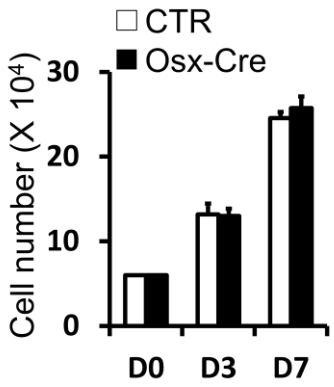

Fig S6. Osx-Cre does not affect BMSC proliferation.

BMSCs were isolated from femur as described in Materials and Methods. Cell numbers were counted at indicated time points. $n = 3$.

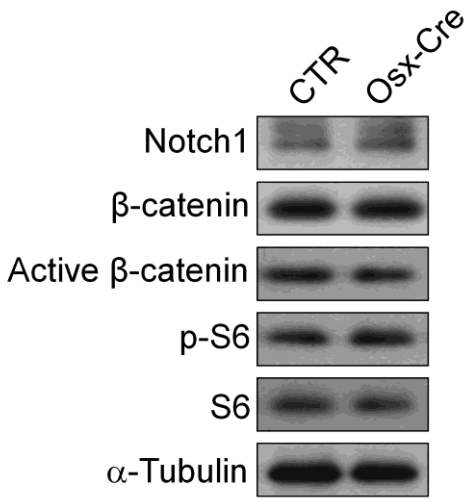

Fig S7. Osx-Cre does not affect β-catenin and Notch 1 level in BMSCs.

BMSCs were isolated from long bones and analyzed by western blotting with indicated antibodies.

Supplemental Table S1

Primer sequences used for qPCR.

	Sense (5'-3')	Antisense (5'-3')
Alpl	GGACAGGACACACACACACA	CAAACAGGAGAGCCACTTCA
Bsp	ACAATCCGTGCCACTCACT	TTTCATCGAGAAAGCACAGG
Col1a1	GAGCCTGAGTCAGCAGATTG	CCAGTACTCTCCGCTCTTCC
Ocn	TGAGCTTAACCCTGCTTGTG	TAGGGCAGCACAGGTCCTA
Runx2	AGGGACTATGGCGTCAAACA	GGCTCACGTCGCTCATCTT
Osx	GGTCCCCAGCTCGAGGAT	CTAGAGCCGCCAAATTTGCT
Opg	TGTGTGTCCCTTGCCCTGACCA	ACACTCGGTTGTGGGTGCGG
Rankl	CAGCATCGCTCTGTTCCTGTA	CTGCGTTTTCATGGAGTCTCA
Mcsf	CCCACATCCCTGAGTCTGTC	GTTCCACCTGTCTGTCCTCA
Pparg	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
Fabp4	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Adipoq	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
Cebpα	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
Cebpβ	ACGACTTCCTCTCCGACCTCT	CGAGGCTCACGTAACCGTAGT
Zfp423	GGTCAGGCTTGATGTCAATGG	TCAGCACTCTCGAACTTCACG
Axin2	CTGCTGACTTAAGAGAGACCAAG	GAAAGTCCGGAAG AGGTATG
Cnx43	TGGGGGAAAGGCGTGAGGGA	ACCCATGTCTGGGCACCTCTCTT
Lef1	TCTCAAGGACAGCAAAGCTC	CACTTGAGGCTTCATGCACAT
Hey1	GAGAAGCAGGGATCTGCTAA	CCCAAACTCCGATAGTCCAT
Jagged1	AGAAGTCAGAGTTCAGAGGCGTCC	AGTAGAAGGCTGTCACCAAGCAAC
18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG