

Supplemental data

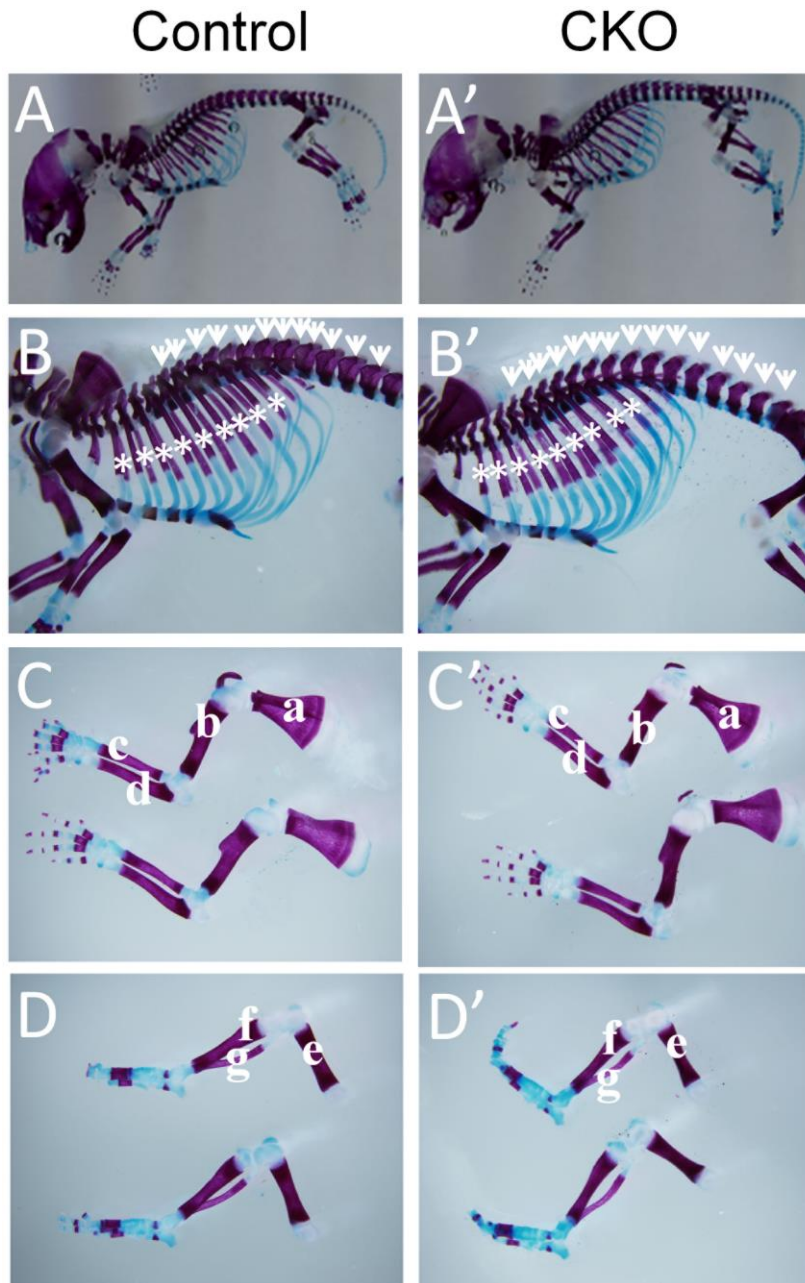


Figure S1. TSC1 deletion by Osx-Cre does not affect embryonic skeleton development. Skeleton preparation of *Tsc1*^{F/F} (Control) (A-D) and *Tsc1*^{F/F};Osx-Cre (CKO) (A'-D') mice at birth. (A, A') Overall staining. (B, B') Thoracic cage (arrow heads point to vertebrae and * indicates rib). (C, C')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

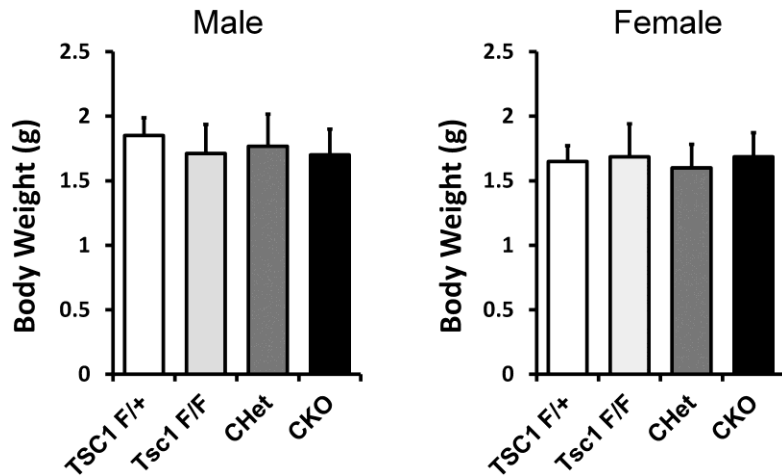


Figure S2. *Tsc1* deletion by *Osx*-Cre did not affect body weight in neonatal mice. Body weight of *Tsc1*^{F/+}, *Tsc1*^{F/F}, *Tsc1*^{F/+};Osx-Cre (CHet) and *Tsc1*^{F/F};Osx-Cre (CKO) mice at one-day old. n=6 for *Tsc1*^{F/+}, n=8 for *Tsc1*^{F/F}, n=6 for CHet, and n=8 for CKO male mice. n=6 for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

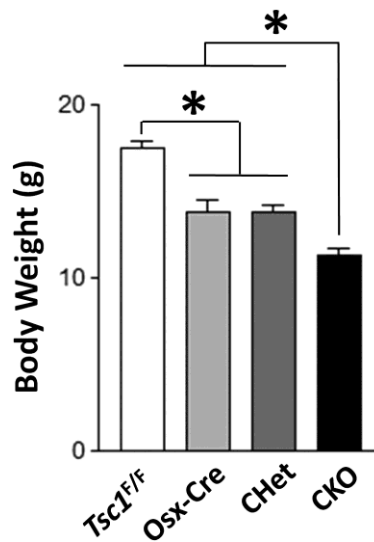


Figure S3. *Tsc1* deletion by *Osx*-Cre leads to decreased body weight at one month old. Body weight of *Tsc1*^{F/F}, Osx-Cre, *Tsc1*^{F/+};Osx-Cre (CHet) and *Tsc1*^{F/F};Osx-Cre (CKO) male mice at one month old. n=41 for *Tsc1*^{F/F}, n=13 for Osx-Cre, n=43 for CHet, and n=45 for CKO mice. Data were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

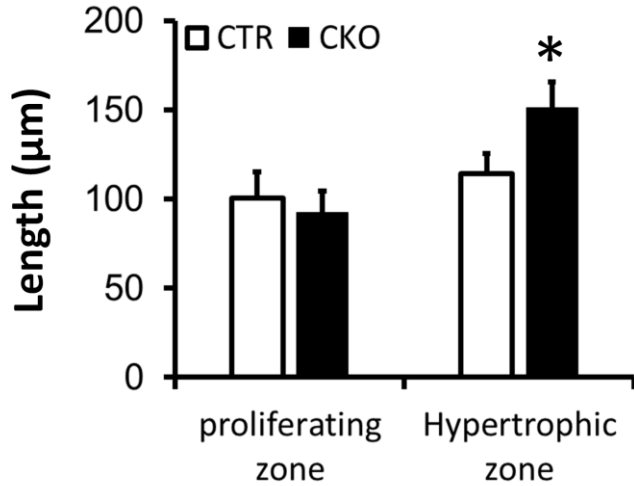


Figure S4. *Tsc1* deletion by *Osx*-Cre leads to increased length of hypertrophic zone in femoral growth plate. * $p < 0.05$, $n = 5$. Values were presented as mean + SE.

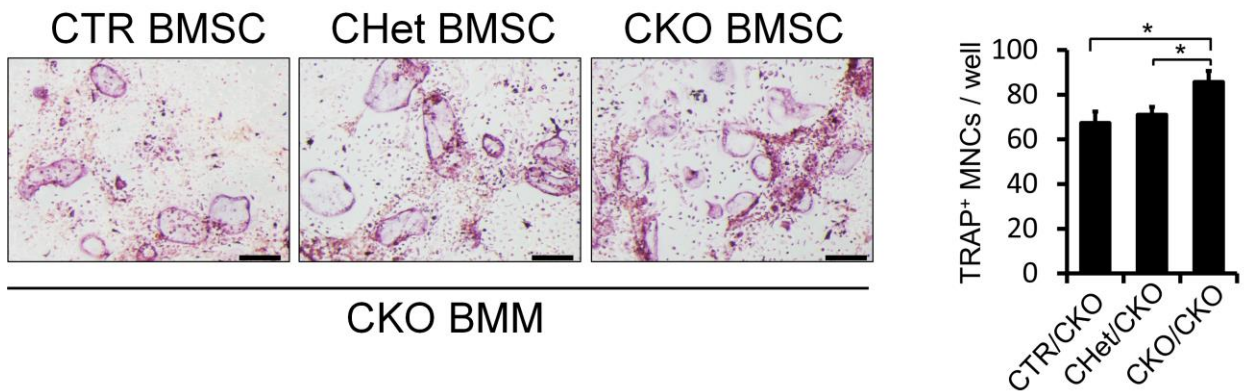


Figure S5. *TSC1*-deficient BMSCs supports osteoclastogenesis

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 $1\alpha, 25$ -dihydroxy vitamin D_3 (Sigma) and 1 μ M prostaglandin E2 for 7 days. Numbers of TRAP-positive (TRAP⁺) multinucleated cells (MNC) (>3 nuclei) were counted (right panel). Scale bar=100 μ m. * $p < 0.05$, $n = 3$. Values were presented as mean + SE.

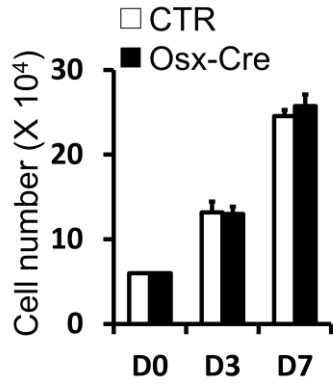


Fig S6. Osx-Cre does not affect BMSC proliferation.

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

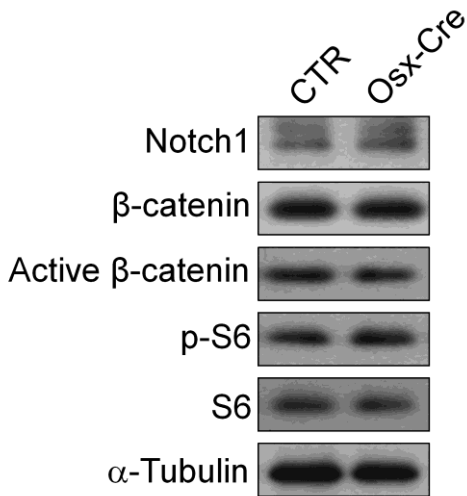


Fig S7. Osx-Cre does not affect β -catenin and Notch 1 level in BMSCs.

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')
<i>Alpl</i>	GGACAGGACACACACACACA	CAAACAGGAGAGCCACTTCA
<i>Bsp</i>	ACAATCCGTGCCACTCACT	TTTCATCGAGAAAGCACAGG
<i>Col1a1</i>	GAGCCTGAGTCAGCAGATTG	CCAGTACTCTCCGCTCTTCC
<i>Ocn</i>	TGAGCTTAACCCTGCTTGTG	TAGGGCAGCACAGGTCCTA
<i>Runx2</i>	AGGGACTATGGCGTCAAACA	GGCTCACGTCGCTCATCTT
<i>Osx</i>	GGTCCCCAGCTCGAGGAT	CTAGAGCCGCCAAATTTGCT
<i>Opg</i>	TGTGTGTCCCTTGCCCTGACCA	ACACTCGGTTGTGGGTGCGG
<i>Rankl</i>	CAGCATCGCTCTGTTCCTGTA	CTGCGTTTTTCATGGAGTCTCA
<i>Mcsf</i>	CCCACATCCCTGAGTCTGTC	GTTCCACCTGTCTGTCCTCA
<i>Pparg</i>	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
<i>Fabp4</i>	ACACCGAGATTTCTTCAAACCTG	CCATCTAGGGTTATGATGCTCTTCA
<i>Adipoq</i>	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
<i>Cebpα</i>	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
<i>Cebpβ</i>	ACGACTTCCTCTCCGACCTCT	CGAGGCTCACGTAACCGTAGT
<i>Zfp423</i>	GGTCAGGCTTGATGTCAATGG	TCAGCACTCTCGAACTTCACG
<i>Axin2</i>	CTGCTGACTTAAGAGAGACCAAG	GAAAGTCCGGAAG AGGTATG
<i>Cnx43</i>	TGGGGGAAAGGCGTGAGGGA	ACCCATGTCTGGGCACCTCTCTT
<i>Lef1</i>	TCTCAAGGACAGCAAAGCTC	CACTTGAGGCTTCATGCACAT
<i>Hey1</i>	GAGAAGCAGGGATCTGCTAA	CCCAAACCTCCGATAGTCCAT
<i>Jagged1</i>	AGAAGTCAGAGTTCAGAGGCGTCC	AGTAGAAGGCTGTCACCAAGCAAC
<i>18s</i>	GTAACCCGTTGAACCCCAT	CCATCCAATCGGTAGTAGCG