

Figure S1. Body weight of two-month-old male mice. * $p < 0.05$, $n = 9-12$. Data were presented as mean + SE.

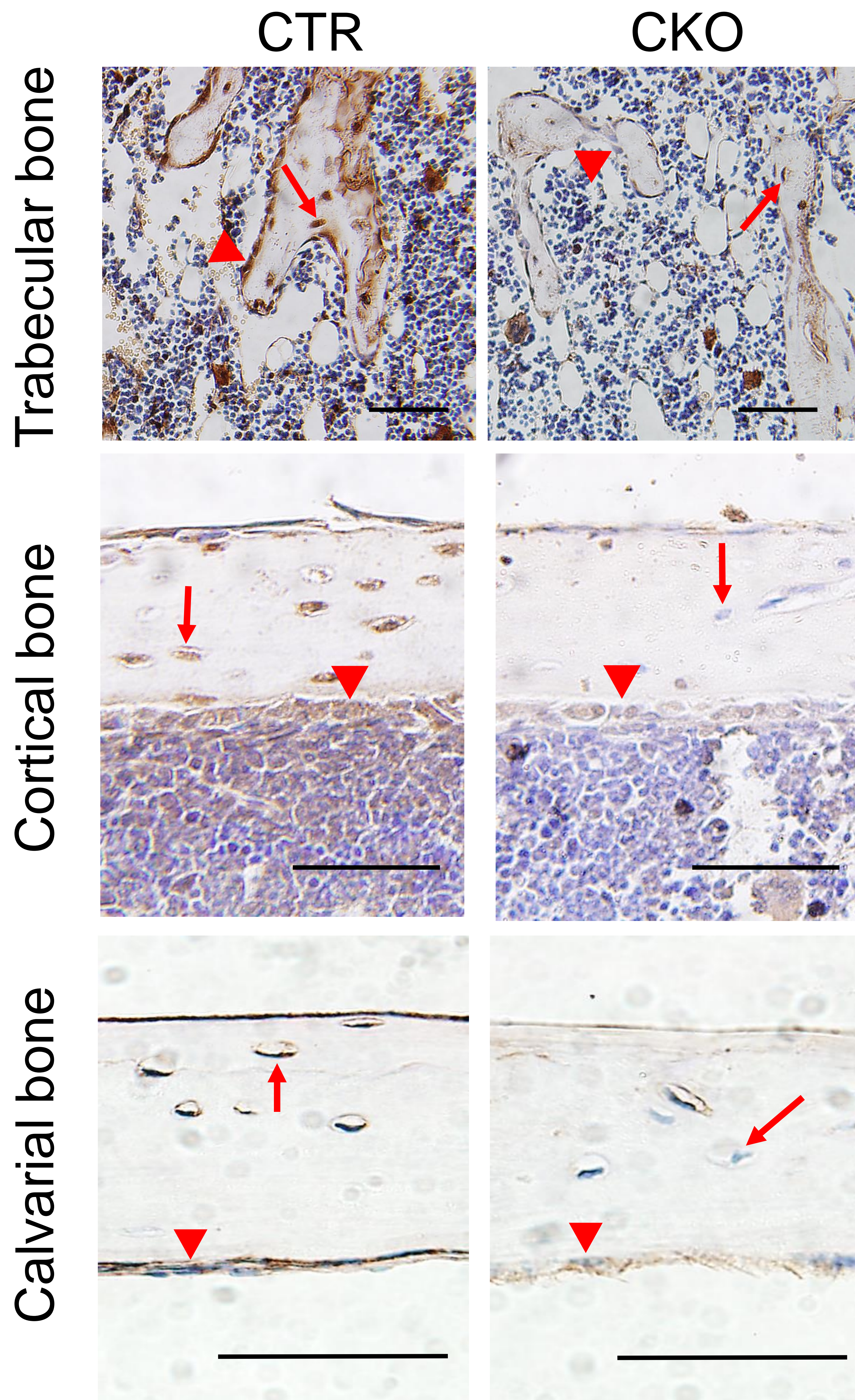


Figure S2. Immuno-staining of FAK in femoral trabecular bone, femoral cortical bone, and calvaria of two-month-old CTR and CKO mice. Arrow heads point to representative osteoblasts and arrows point to representative osteocytes. Dark brown color represents positive staining. Scale bar=40mm.

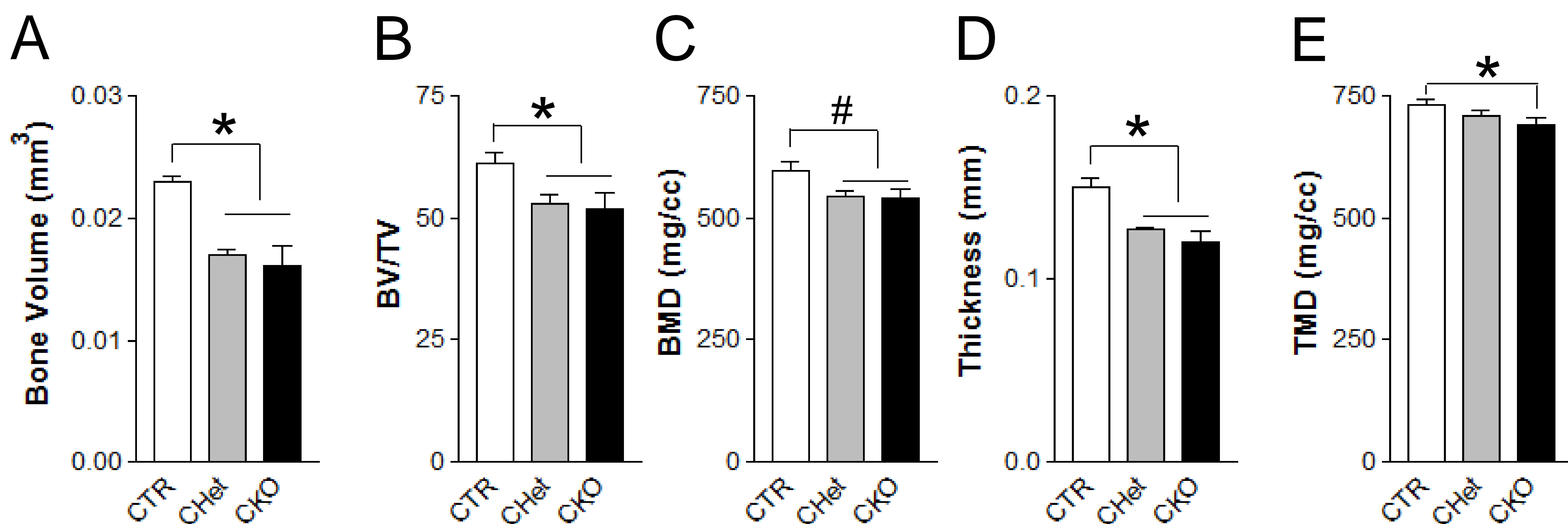


Fig S3. FAK deletion led to decreased frontal bone mass. MicroCT

measurements were performed in frontal bones of 2-month-old male $FAK^{F/F}$ (CTR), $FAK^{F/+};Osx-Cre$ (CHet), and $FAK^{F/F};Osx-Cre$ (CKO) mice. Bone volume (A); BV/TV, bone volume per tissue volume (B); BMD, bone mineral density (C); frontal thickness (D); TMD, tissue mineral density (E). * $p < 0.05$, # $0.05 < p < 0.1$, $n = 5-6$ per group. Values were presented as mean + SE.

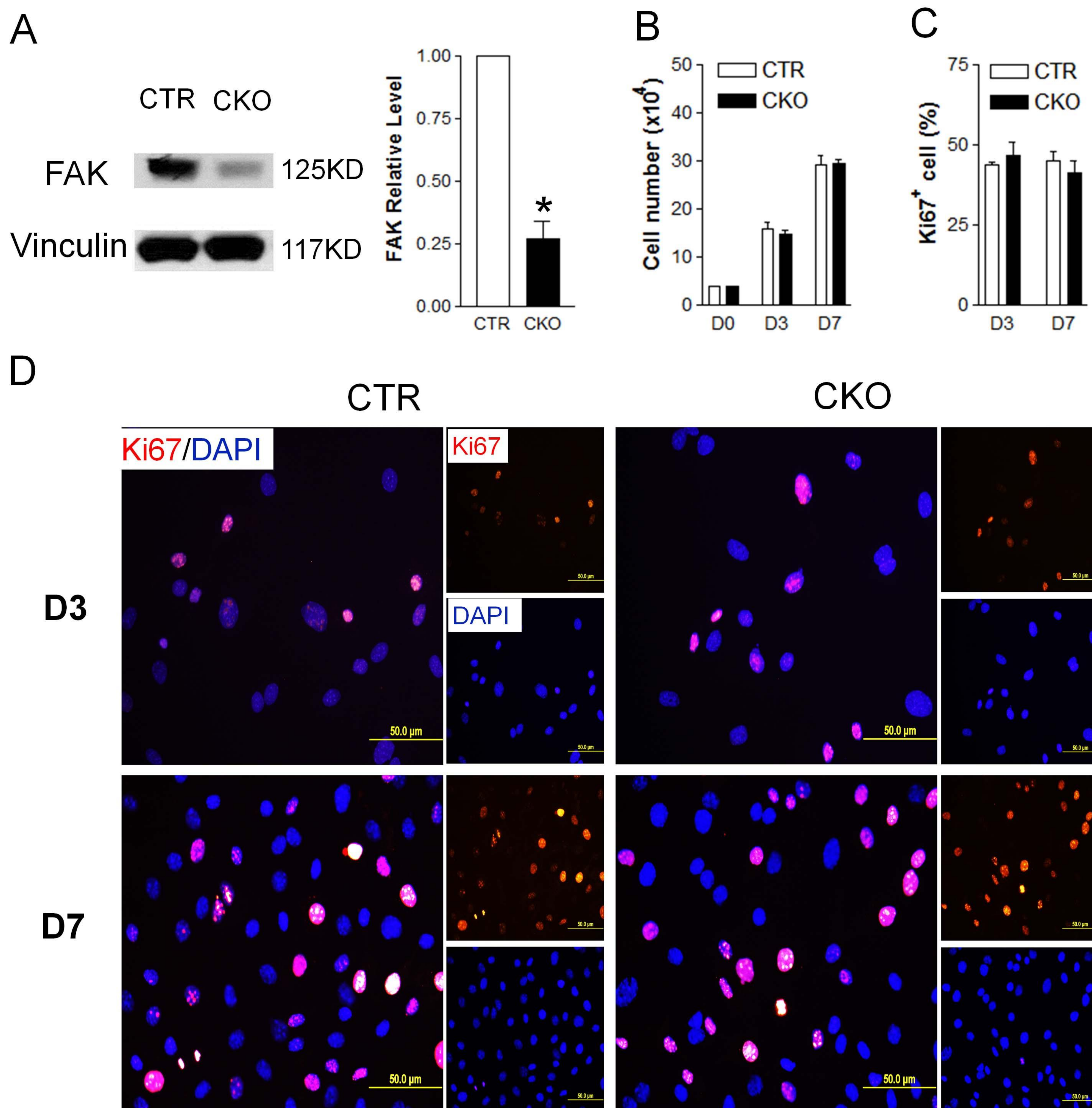


Figure S4. FAK deletion doesn't affect the proliferation of calvarial osteoblasts. (A) FAK expression (left panel) and quantification (right) analyzed by western blot in primary calvarial osteoblasts isolated from CTR and CKO neonatal mice ($n = 5$ per group). (B) Cell numbers and (C) Percentage of Ki67 positive cells during calvarial osteoblasts culture in osteogenic medium for 3 and 7 days ($n=3$). (D) Representative fluorescent images of Ki67 (Red) and Dapi (blue) staining in FAK CTR and CKO calvarial osteoblast at 3rd and 7th day, in the same cultures system as described in B. * $p<0.05$. Data were presented as mean + SE.

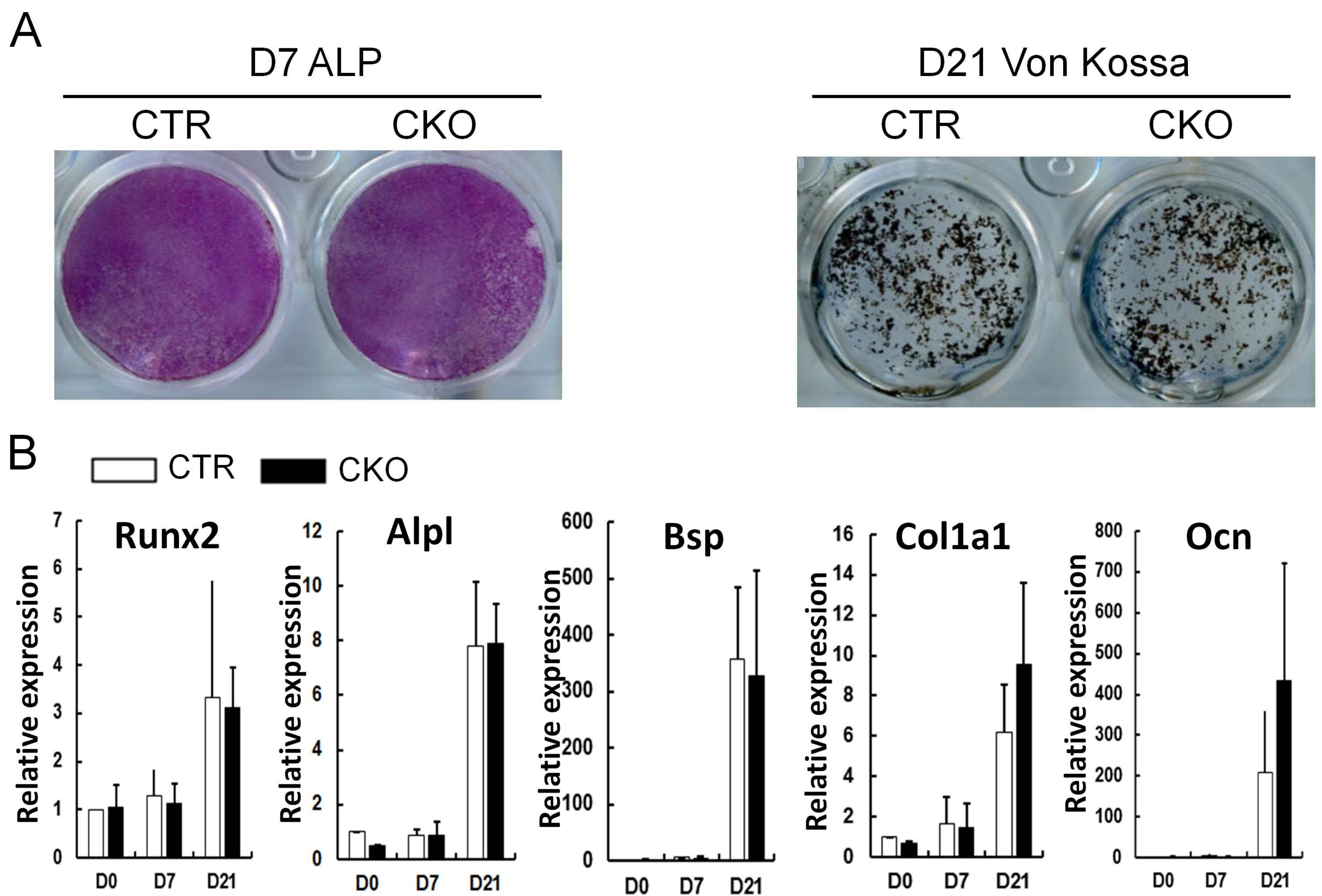


Figure S5. Osteoblast differentiation is not compromised by FAK deletion

in calvarial osteoblasts. (A) Alkaline phosphatase (ALP) staining at day 7

culture (left) and Von Kossa staining (right) at day 21 osteogenic culture of

FAK CTR and CKO primary calvarial cells, in the cultures described in Figure

S4. Images were the representatives of six independent experiments. (B)

Quantitative-PCR analysis of the mRNA expression of osteoblast

differentiation marker genes at indicated time-points during osteogenic

differentiation (n=3). Bars represent mean + SE.

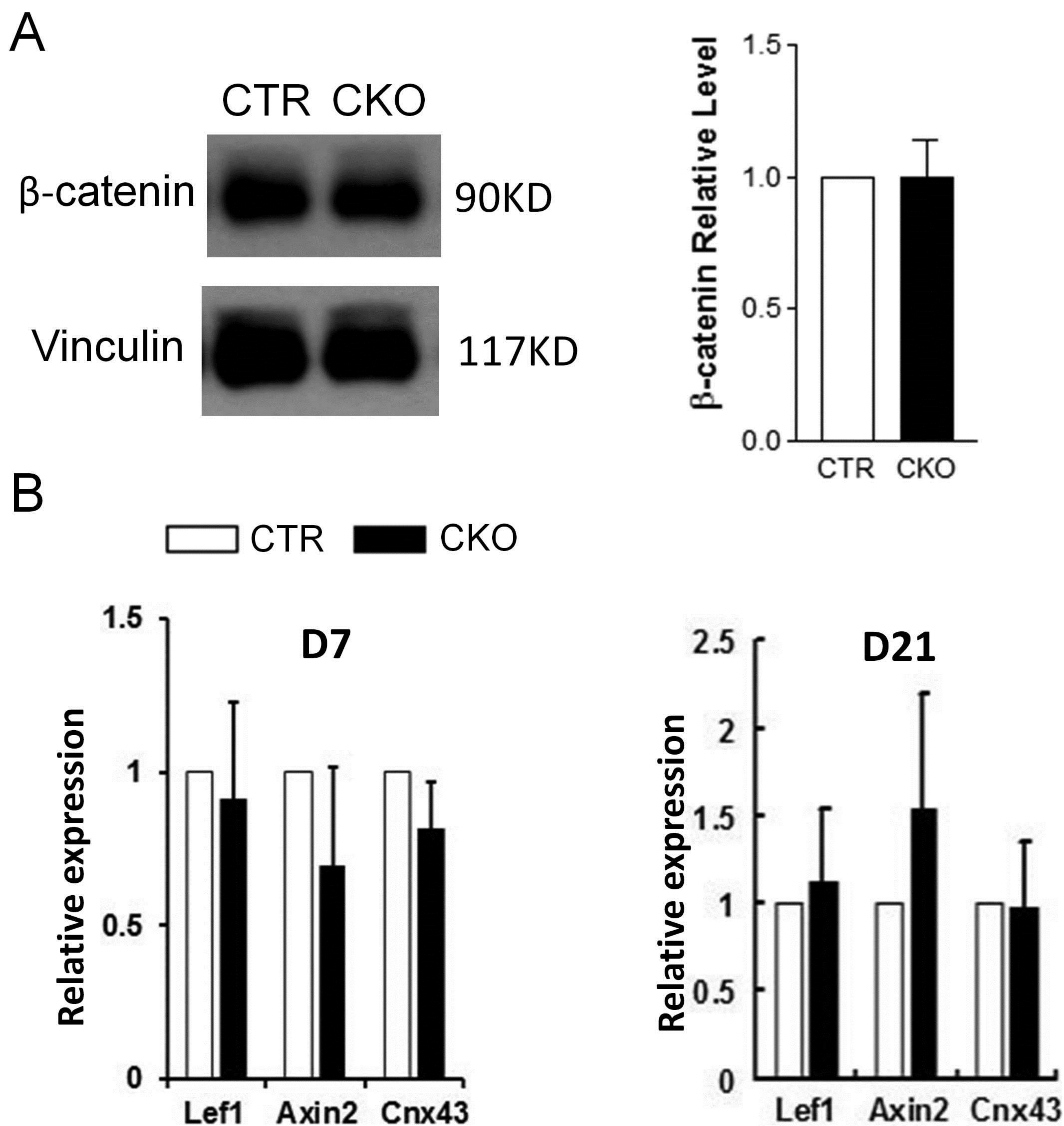


Figure S6. Wnt/ β -catenin signaling is not altered in FAK deficient calvarial osteoblasts. (A) β -catenin expression (left panel) and quantification (right) analyzed by western blot in primary calvarial osteoblasts isolated from CTR and CKO neonatal mice ($n = 7$ per group), in the cultures described in Figure S4. (B) Quantitative-PCR analysis of the mRNA expression of Wnt/ β -catenin target genes at indicated time-points during osteogenic differentiation ($n=3$).

Bars represent mean + SE.