Supplemental information

Toll-like receptor 4 signaling

in osteoblasts is required for load-induced

bone formation in mice

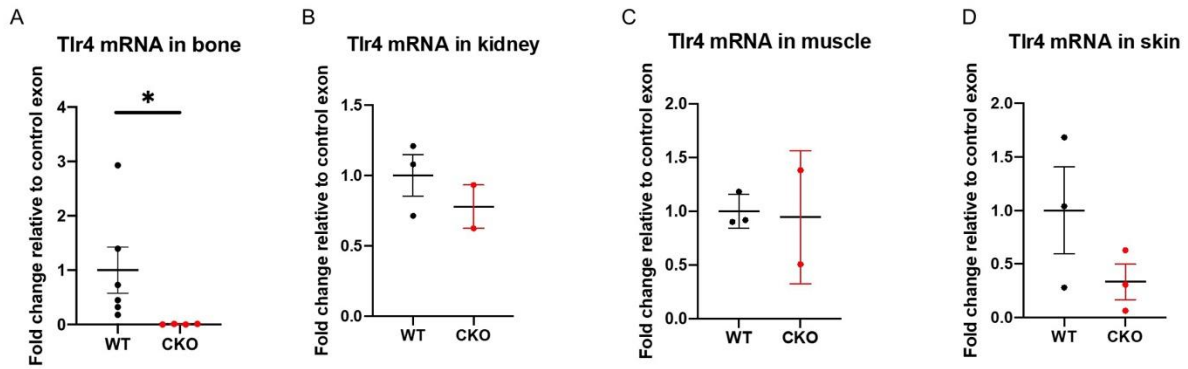Ibtesam Rajpar, Gaurav Kumar, Paolo Fortina, and Ryan E. Tomlinson

Fig. S1. Tlr4 mRNA. A) Tlr4 mRNA expression is significantly decreased in the ulna bones of CKO mice as compared to WT mice. There were no significant differences in Tlr4 expression in B) kidney, c) muscle, or d) skin (adjusted $p \leq 0.05$ by t-test). Data are represented as mean ± SEM. **Related to Fig. 2.**

WT CKO

Fig. S2. Skeletal preparation of postnatal D0 mice. No differences were noted in the size or shape of skeletal elements in WT (Tlr4$^{fl/fl}$) and CKO (Tlr4$^{fl/fl}$; OC-Cre+) mice. **Related to Fig. 2.**
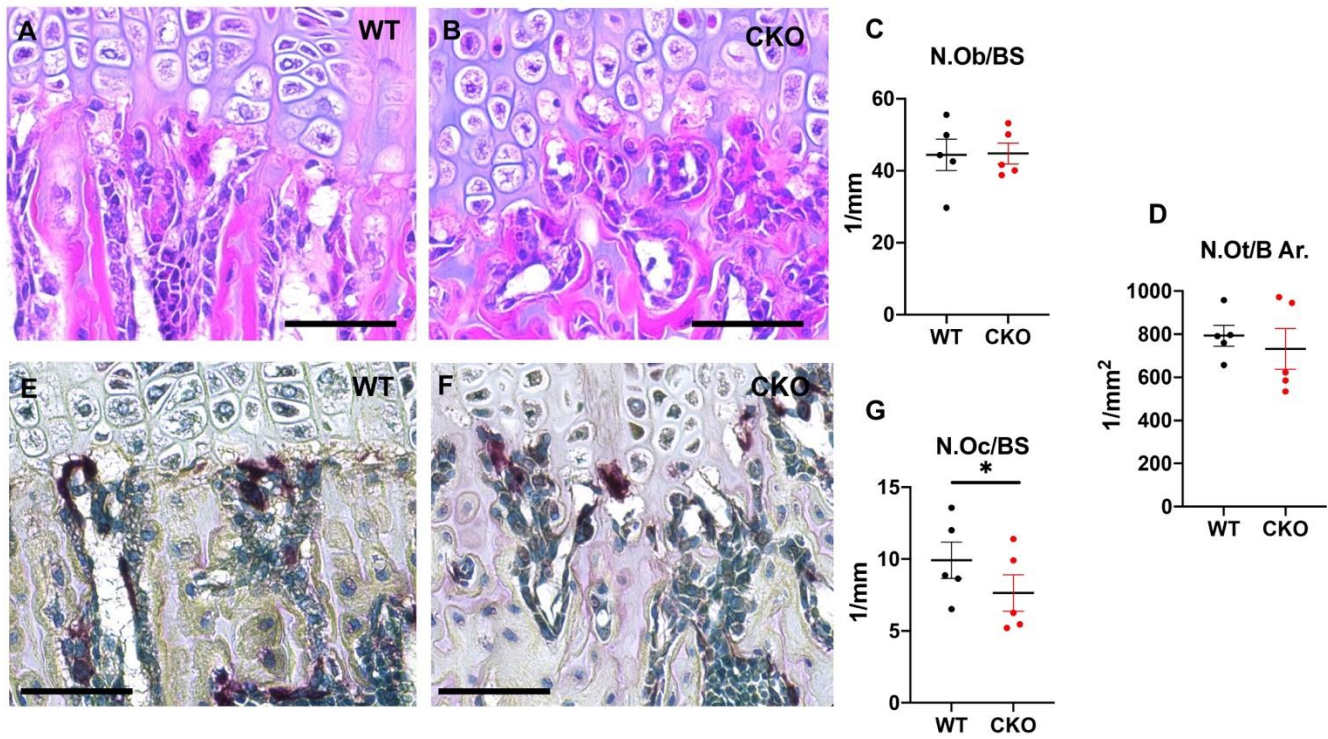
Fig. S3. Quantification of bone cells in young mice. Longitudinal sections of femurs from WT and CKO mice were stained using A, B) H&E to quantify C) osteoblasts per bone surface and D) osteocytes per bone area, or E,F) TRAP stain to quantify G) osteoclasts per bone surface in trabecular bone. Scale bars are 50 microns. * denotes significant differences by t-test (adjusted p ≤ 0.05 by t-test). Data are represented as mean ± SEM. **Related to Fig. 2.**
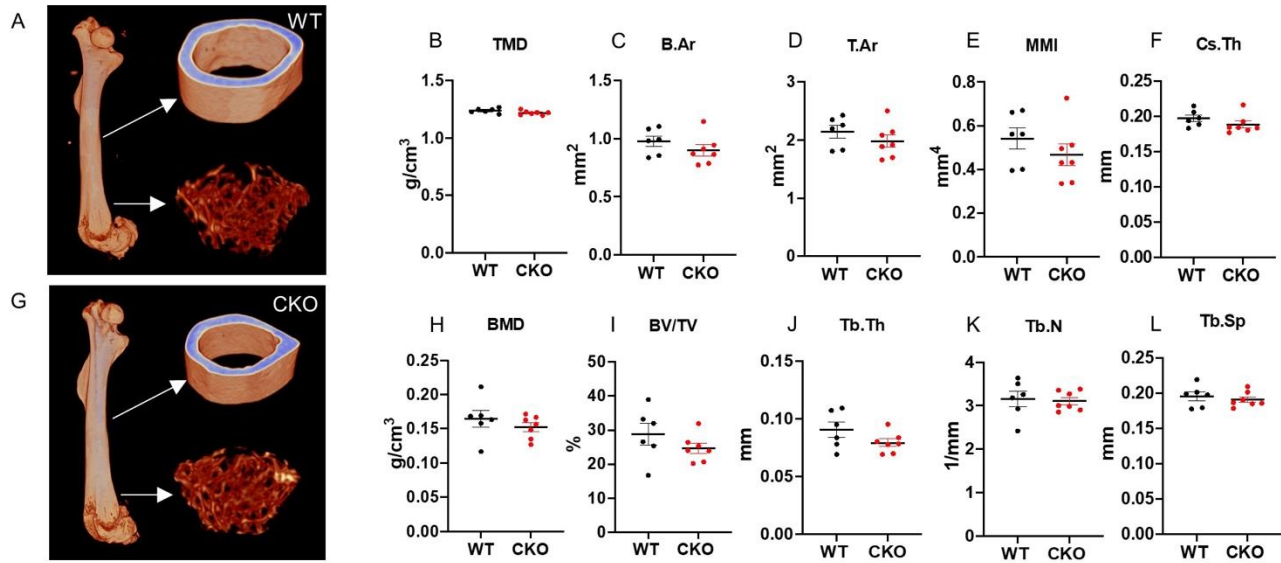
Fig. S4. Quantification of cortical and trabecular bone parameters in adult male femurs. White arrows in reconstructed images indicate ROI for cortical and trabecular bone in A) WT and G) CKO femurs. No differences were noted in cortical (B-F) or trabecular (H-L) bone parameters between WT and CKO mice. Data are represented as mean ± SEM. **Related to Fig. 2.**
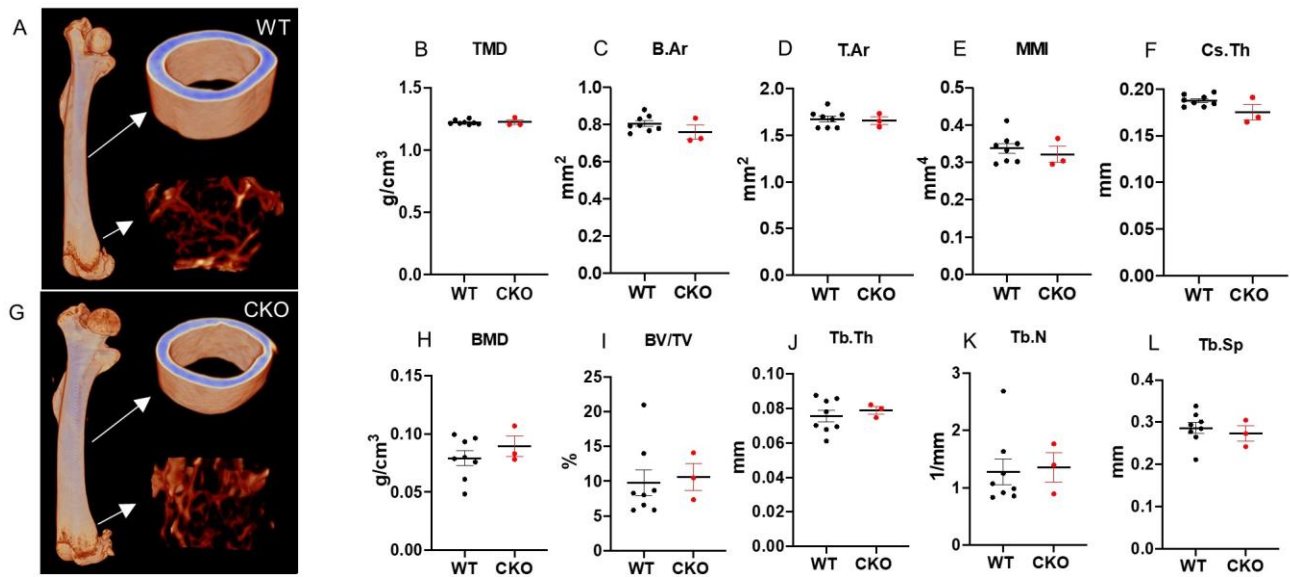
Fig. S5. Quantification of cortical and trabecular bone parameters in adult female femurs. White arrows in reconstructed images indicate ROI for cortical and trabecular bone in A) WT and G) CKO femurs. No differences were noted in cortical (B-F) or trabecular (H-L) bone parameters between WT and CKO mice. Data are represented as mean ± SEM. **Related to Fig. 2.**
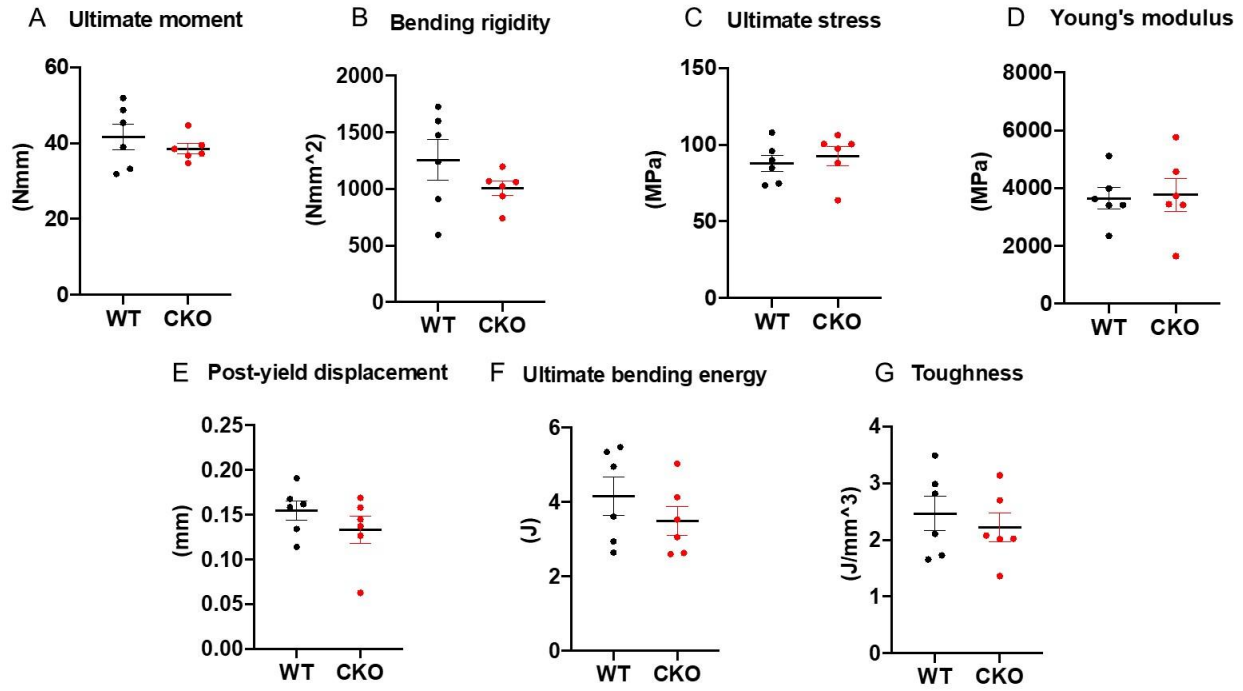
Fig. S6. Mechanical testing of adult mouse femurs. A-G) Femurs from WT and CKO mice were subjected to standard three-point bending and data were analyzed using a custom GNU Octave script to derive structural and material properties. Data are represented as mean ± SEM. **Related to Fig. 2.**
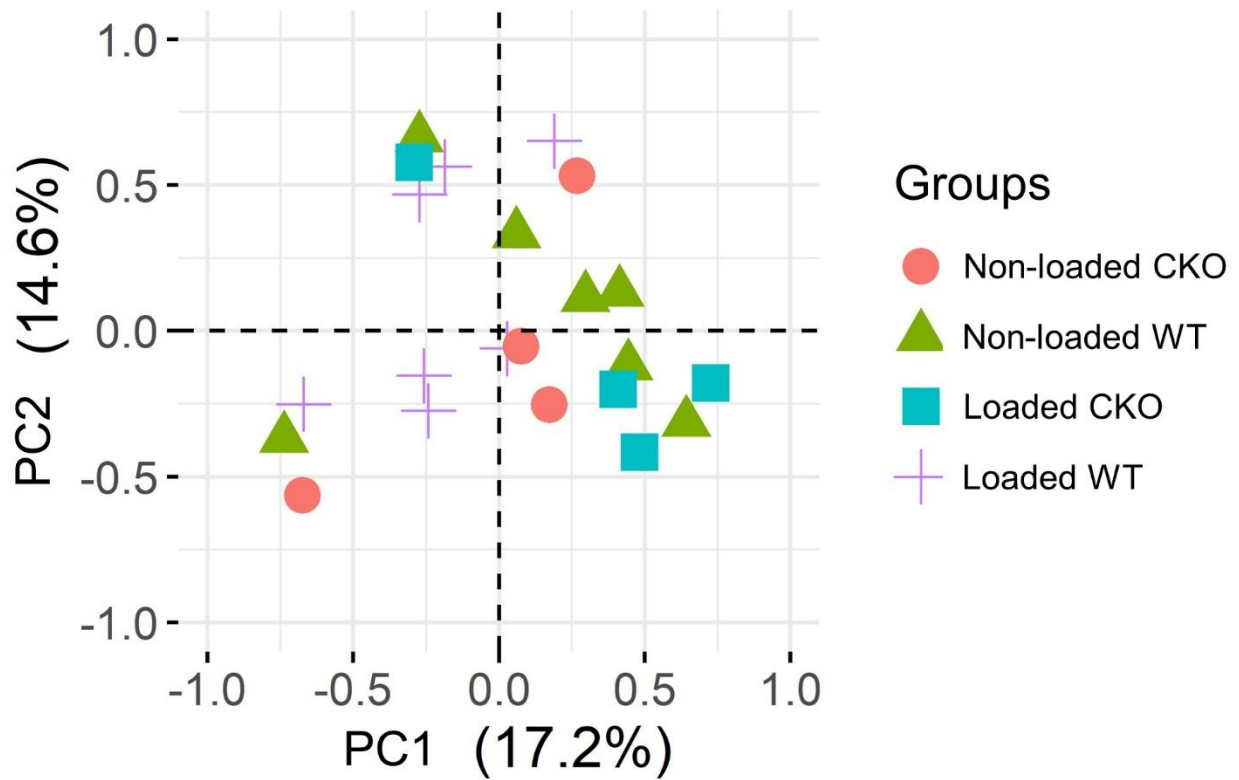
Fig. S7. Principal component analysis. RNA sequencing was performed on wildtype and CKO load and non-loaded forelimbs. PC1 and PC2 together accounted for 31.8% of the variance, with some clustering within groups but relatively little separation between groups. **Related to Fig. 4,5.**
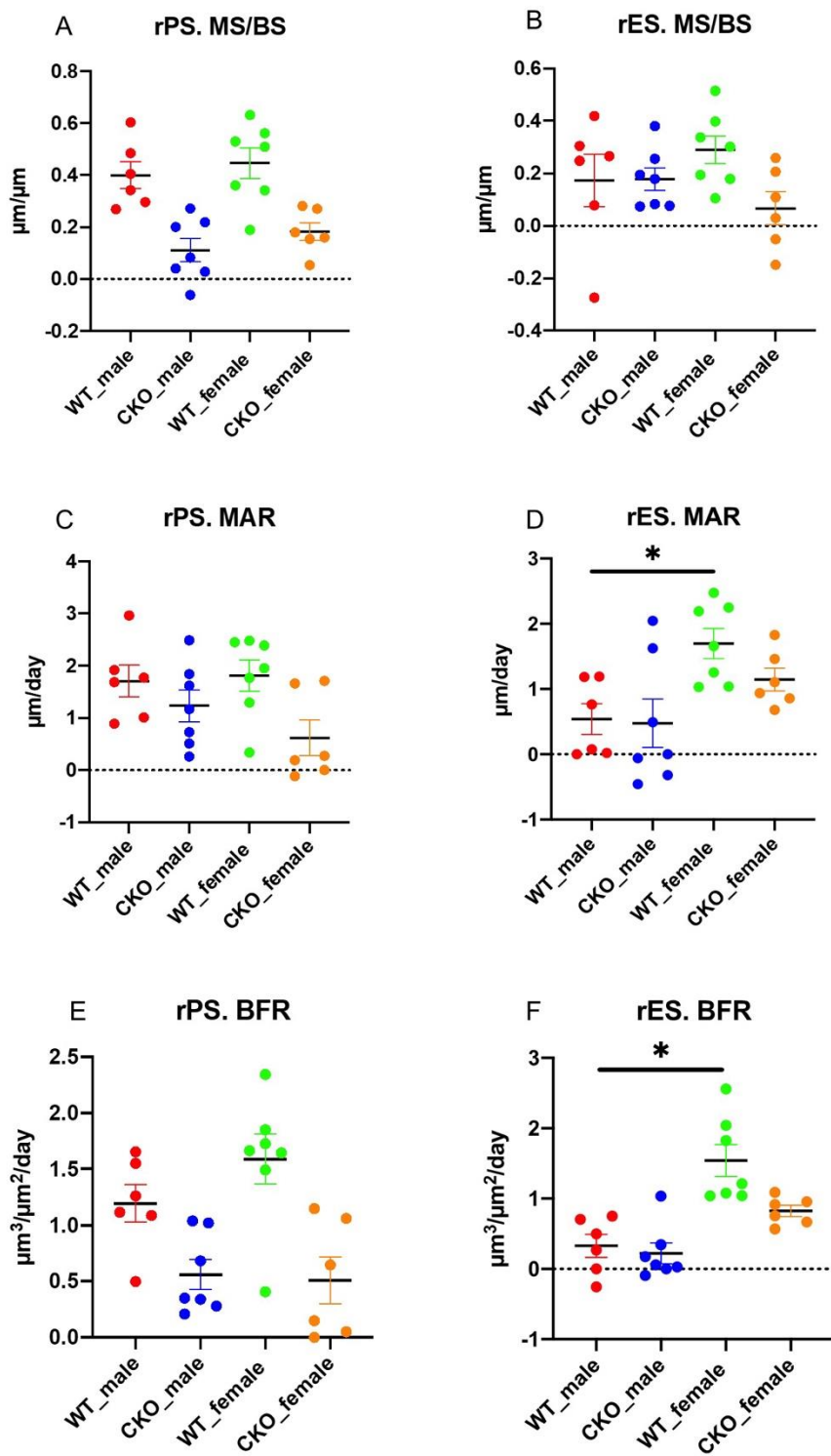
Fig. S8. Comparison of load-induced bone formation parameters between male and female mice. Loss of osteoblastic *Tlr4* affected load-induced endosteal bone formation in female mice more strongly than male mice (D, F). * denotes significant differences by one-way ANOVA (adjusted p ≤ 0.05). Data are represented as mean ± SEM. **Related to Fig. 2.**

Table S1. Differentially expressed genes. **Related to Fig. 4,5**.

Table S2. Gene set enrichment analysis. **Related to Fig. 6.**