492 **Supplemental Figures**

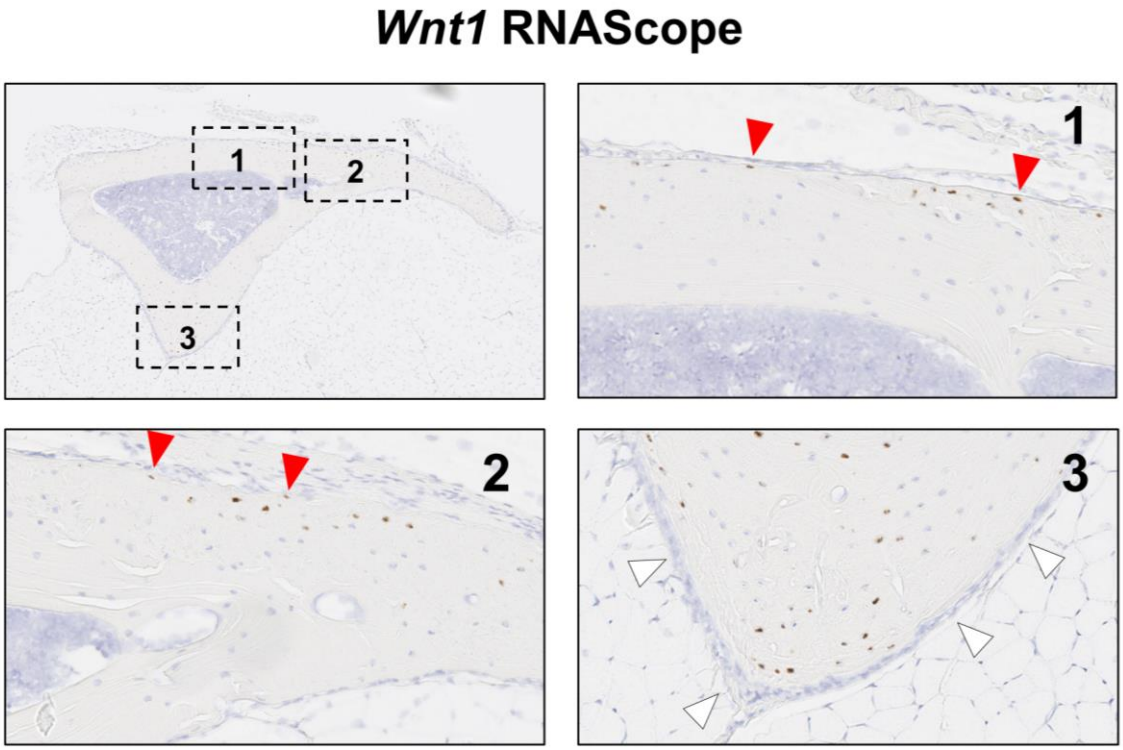

Figure S1. **Supplemental *Wnt1* RNAScope**. Panels 1-3 show *Wnt1* expression in the loaded tibia of a male Wnt1F/F mouse after 5 bouts of loading. *Wnt1* expression was detected in osteocytes on both the tensile and compressive sides of the bone, including osteocytes near the bone surface (red arrowheads), but not in osteoblasts or bone lining cells on the bone surface (white arrowheads). Results representative of n=4.

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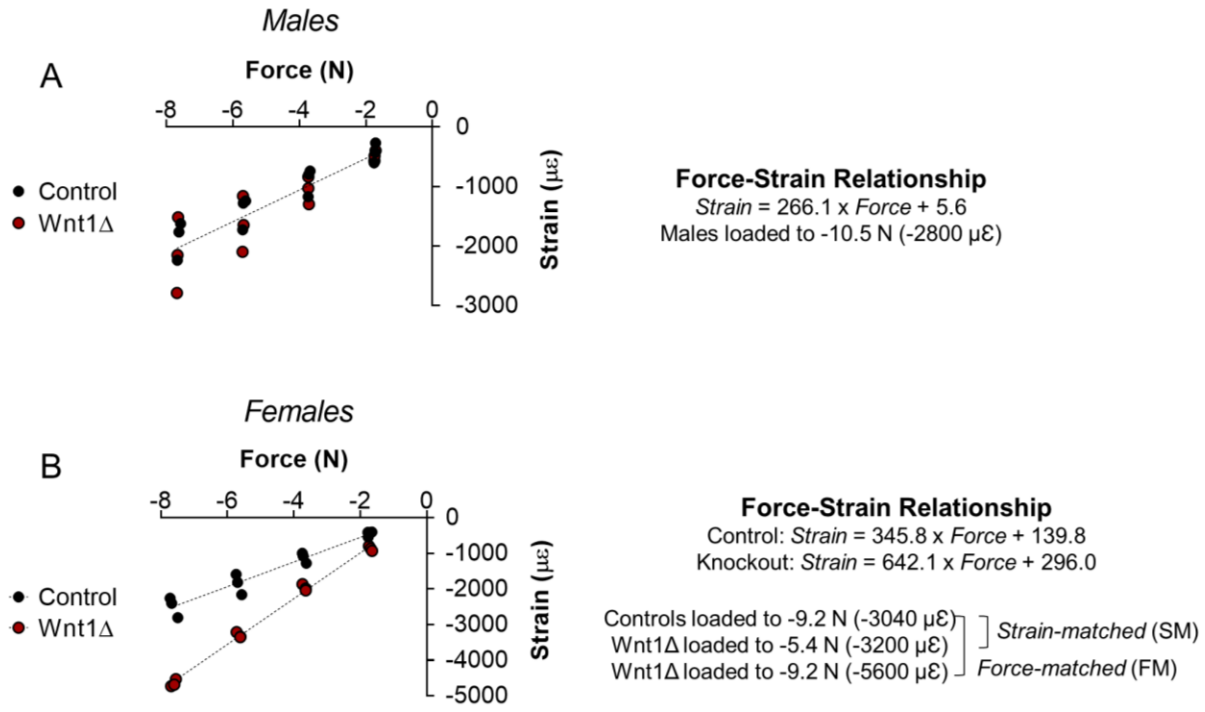


Figure S2. **Force-strain relationship**. Strain gage analysis was performed 3 weeks after tamoxifen induction to experimentally determine the force-strain relationship at the antero-medial surface of the tibia, approximately 5 mm from the tibio-fibrular junction. (A) In males, strain-matched loading was achieved by loading mice to -10.5 N, which engendered a target strain of approximately -3000 με in both groups. (B) In females, strain-matched (SM) loading was achieved by loading controls to -9.2 N (-3040 με) and knockouts to -5.4N (-3200 με). Female knockouts in the force-matched (FM) group were subjected to a peak target strain of -5600 με. n=3/group.

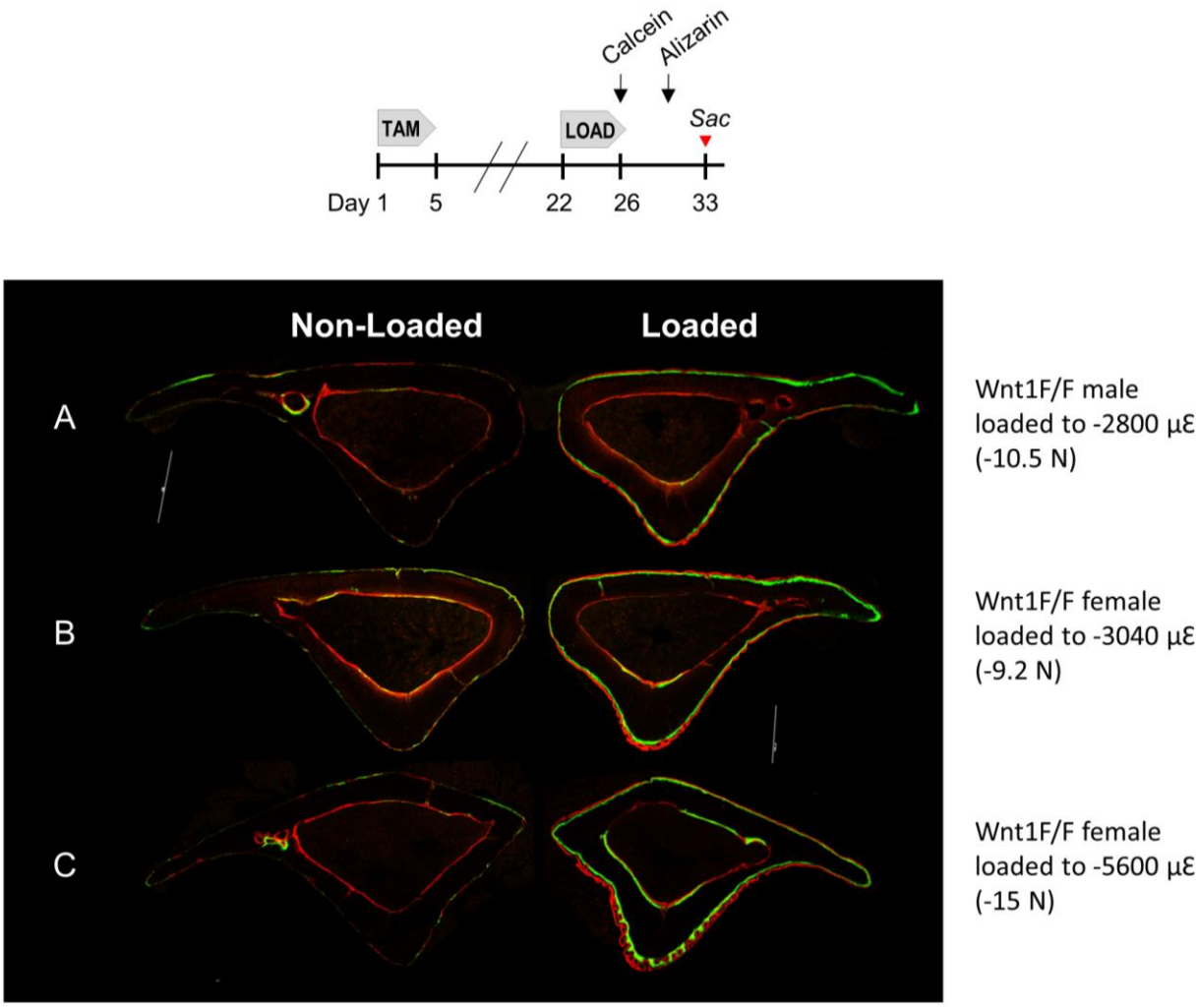


Figure S3. **Representative images of loaded vs non-loaded tibias used for bone formation analysis by dynamic histomorphometry**. Analysis of calcein (green) and alizarin (red) incorporation on the inner (Ec) and outer (Ps) surfaces of the bone was used to calculate % mineralizing surface (% MS/BS), mineral apposition rate (MAR), and bone formation rate (BFR/BS). (A-B) Results representative of the lamellar bone formation response observed in Wnt1F/F mice loaded to -3000 με. (C) In contrast, a woven bone formation response was observed in a Wnt1F/F female loaded to -5600 με.

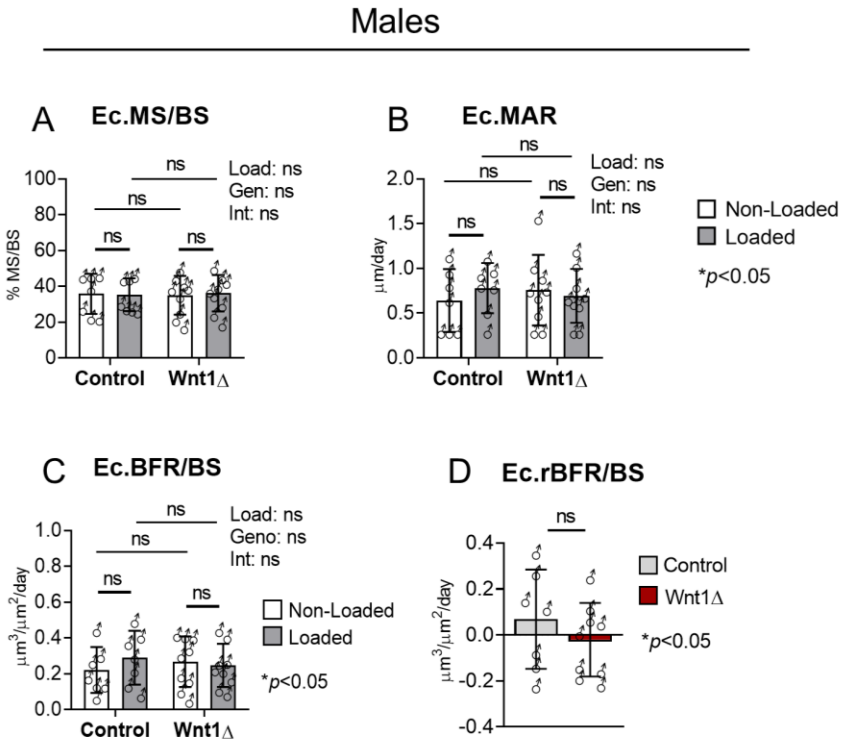
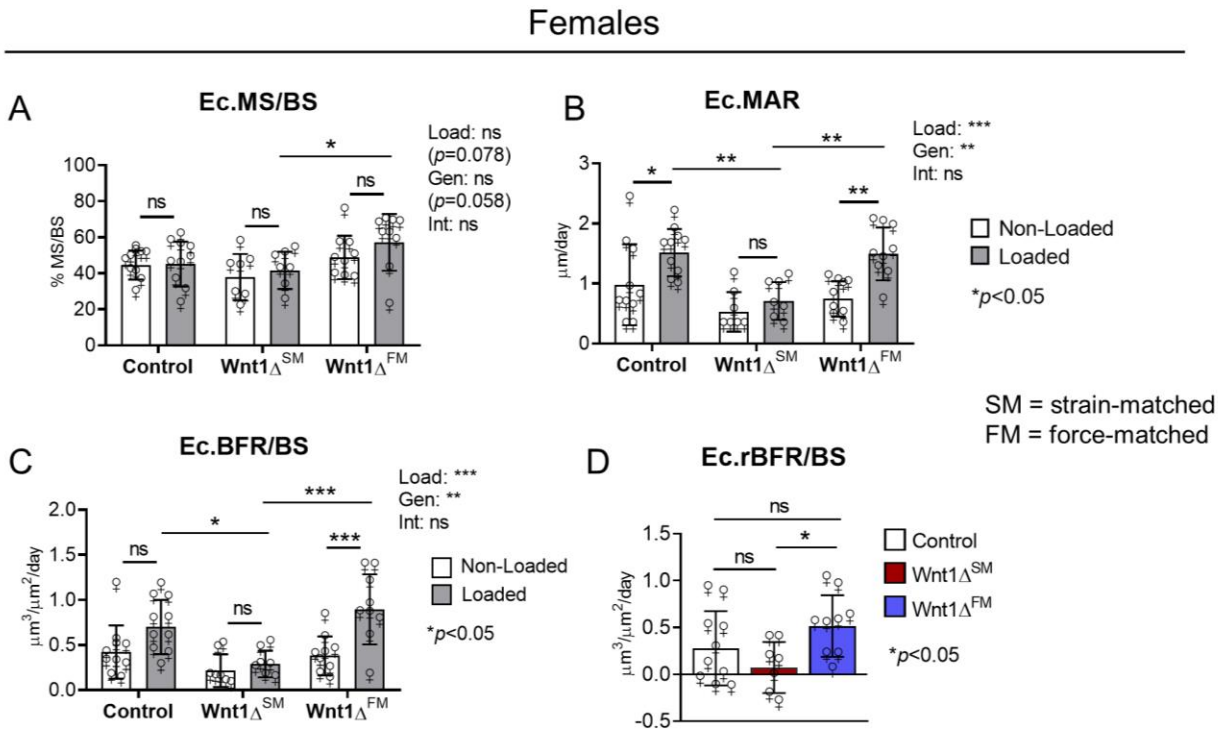


Figure S4. Endocortical bone formation in male Wnt1F/F vs Wnt1F/F; OsxCreERT2 mice. Strain-matched loading (-3040 με; -10.5 N) had a negligible effect on endocortical bone formation in male Wnt1F/F (control) and Wnt1F/F; OsxCreERT2 (knockout) mice. n=9-11/genotype.



<u>Figure S5</u>. Endocortical bone formation in Wnt1F/F vs Wnt1F/F; OsxCreERT2 females. Wnt1F/F (control) females were loaded to -3040 με (-9.2 N), while Wnt1F/F; OsxCreERT2 (knockout) females were subjected to strain-matched (SM) or force-matched (FM) loading by applying a loading force of -5.4 N or -9.2 N, respectively. Strain-matched loading did not elicit a strong endocortical response in either control or knockout mice. n=7-10/group.

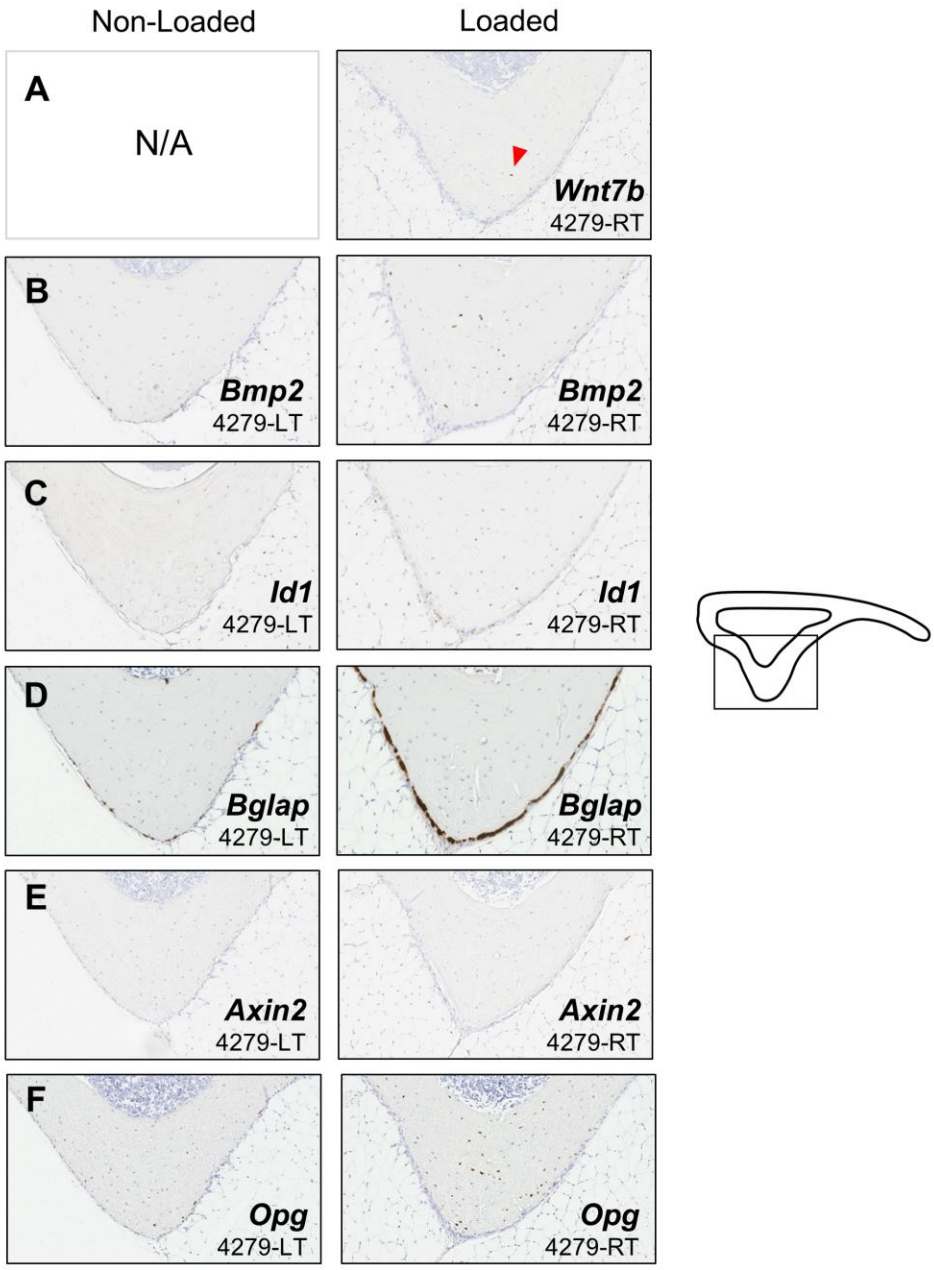


Figure S6. Supplemental gene expression analysis by RNAScope. Gene expression was analyzed in OsxCreERT2-negative Wnt1F/F or Wnt7bF/F mice after 5 days of loading (-3000 με). Loading increased osteo-inductive *Bmp2* expression in the tibia, particularly in osteocytes located at the site of peak compressive strain (B). Expression of Bmp target gene *Id1* was increased on the periosteal surface of loaded bones (C). *Bglap* – a marker of osteoblast maturation/activation – was also induced by loading on the bone surface (D). Wnt target genes were also analyzed histologically by *in situ* hybridization (E-F). Images are from a male Wnt1F/F mouse. Data representative of n=3.

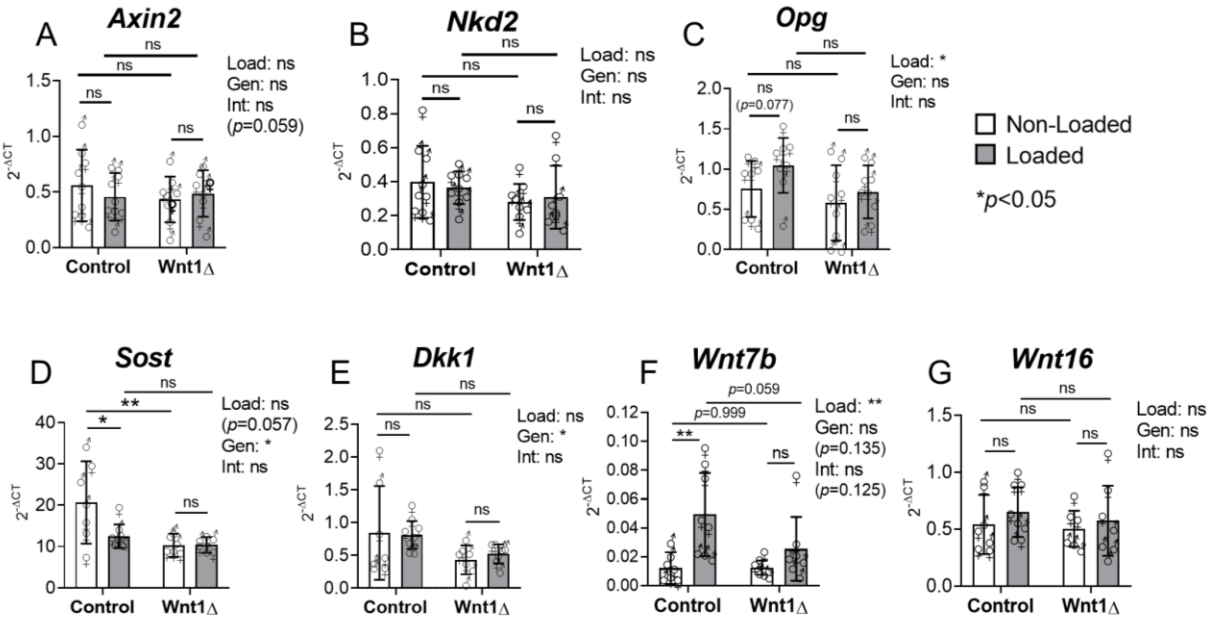
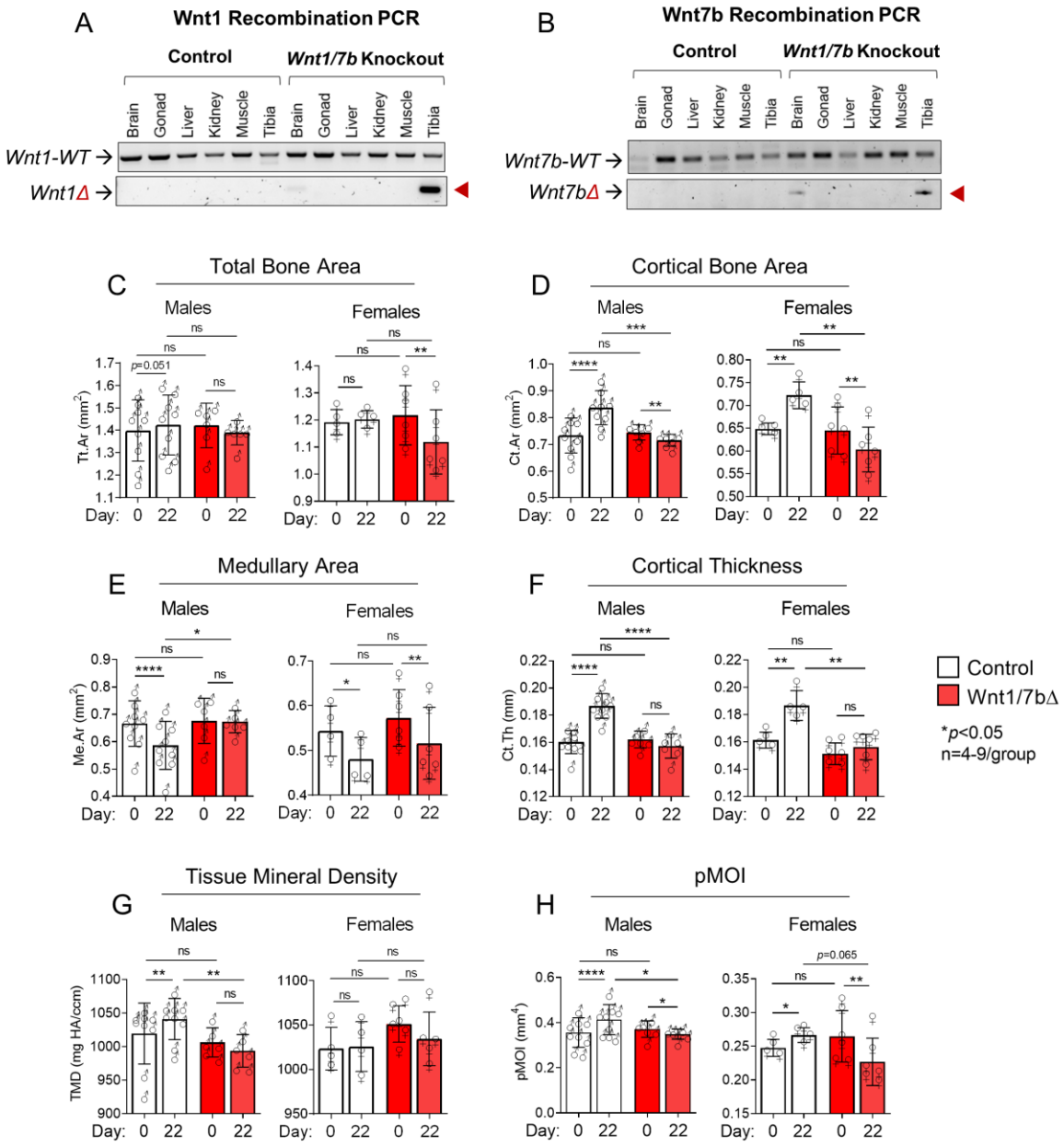


Figure S7. Wnt pathway-related genes. Gene expression was analyzed on day 26 as described in Figure 6. RT-qPCR was used to analyze the expression of Wnt target genes (A-C), Wnt pathway inhibitors (D-E), and bone anabolic *Wnt7b* and *Wnt16* (F-G) in the tibias of Wnt1F/F and Wnt1F/F; OsxCreERT2 mice after 5 days of strain-matched loading (-3000 με). n=6-9/genotype.



<u>Figure S8</u>. The skeletal deficits associated with *Wnt1* deletion were re-capitulated in *Wnt1/7b* knockouts. *Wnt1/7b* double conditional knockout mice were generated by tamoxifen-treating 5-month old Wnt1F/F; Wnt7bF/F; OsxCreERT2 males and females. Tamoxifen-treated Wnt1F/F; Wnt7bF/F mice served as control. (A-B) DNA recombination analysis was performed on Day 22 as described in Figure 2. Bone-specific *Wnt1* (exon 2-4) and *Wnt7b* (exon 3) deletion was observed in *Wnt1/7b* knockout mice. (C-H) Serial µCT analysis was used to evaluate the effect of *Wnt1/7b* deletion on cortical bone morphometry, as described in Figure 3. n=4-9/group.

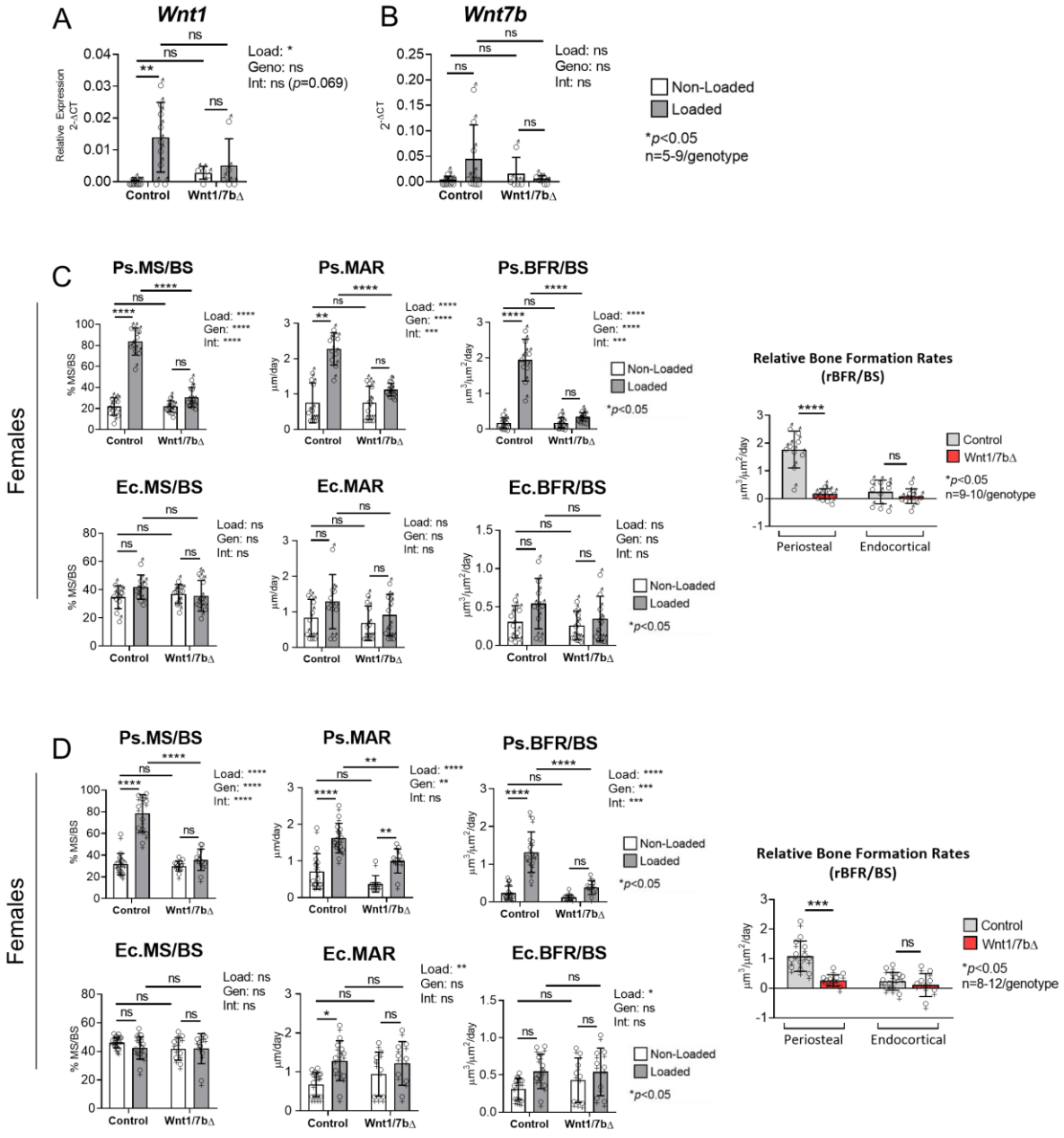


Figure S9. Loading-induced bone formation was reduced in *Wnt1/7b* knockouts. (A-B) Gene expression analysis was performed on Day 26 as described in Figure 6. *Wnt1* expression was assayed using primers that bind in exons 2-3, and *Wnt7b* expression was assayed using primers that bind in exons 2-3. (C-D) Bone formation indices in control vs *Wnt1/7b* knockout male and female mice subjected to 5 days of strain-matched (-3000 με) loading. n=8-12/group.

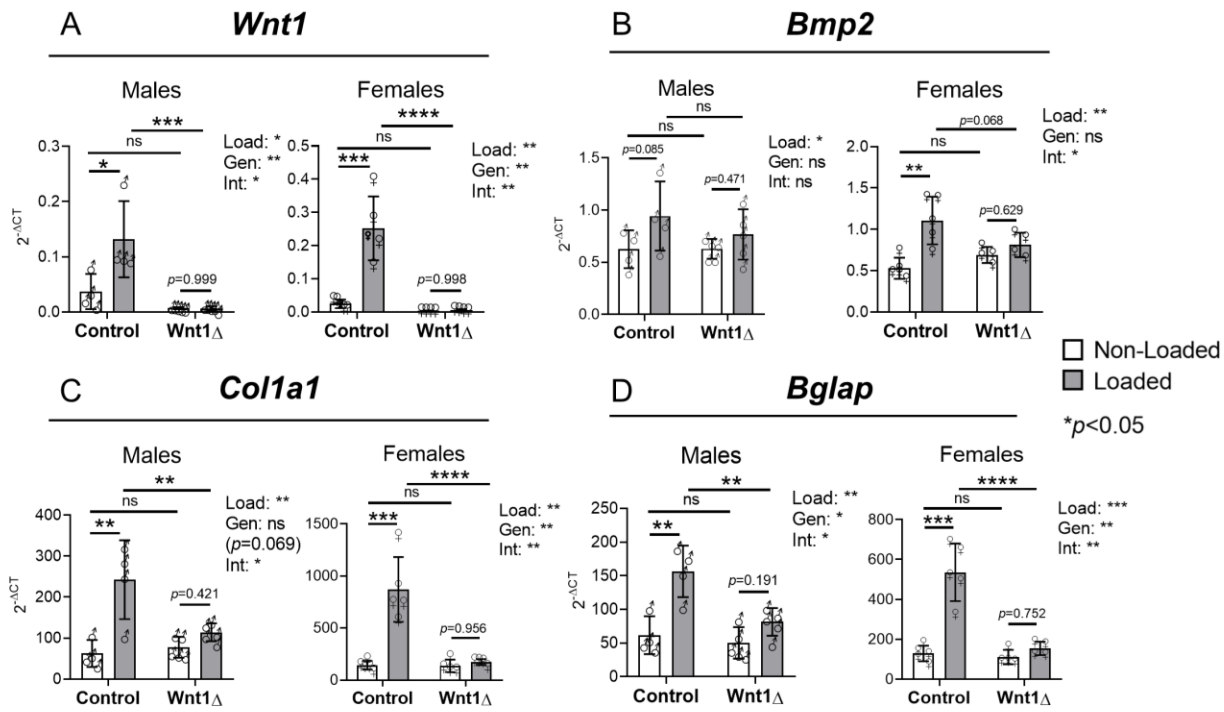


Figure S10. Gene expression in males vs females. Irrespective of sex, loading-induced *Wnt1*, *Bmp2*, *Col1a1*, and *Bglap* upregulation was significant (or near-significant) in control but not knockout mice. In general, the transcriptional response to loading was stronger in Wnt1F/F females compared to Wnt1F/F males, consistent with the finding that Ps.rBFR/BS was higher in female vs male controls.