

Figure S1

1wk

4wk

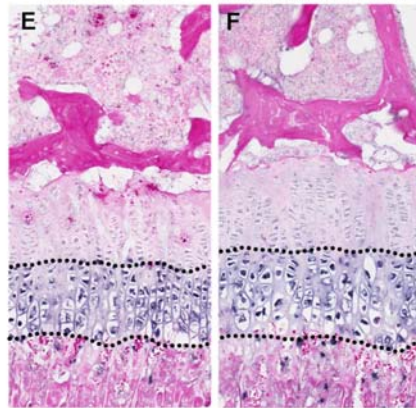
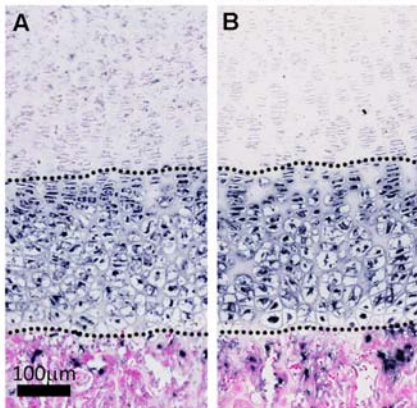
Col2-cre WT

Col2-cre Sox9GFP

Col2-cre WT

Col2-cre Sox9GFP

in situ - Collagen X



Mason-Trochrome Staining

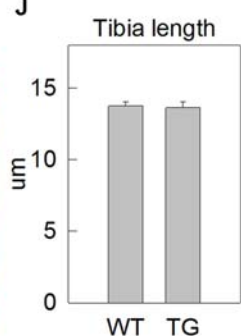
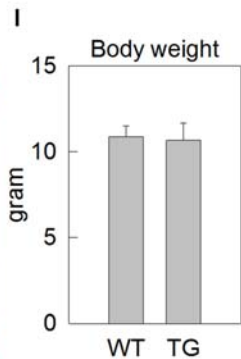
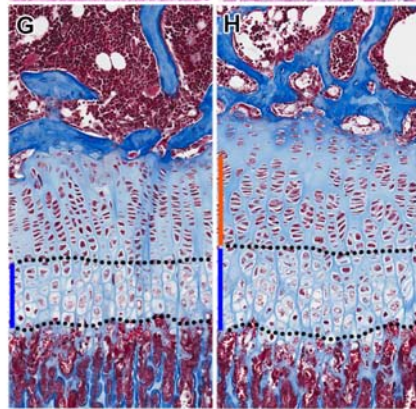
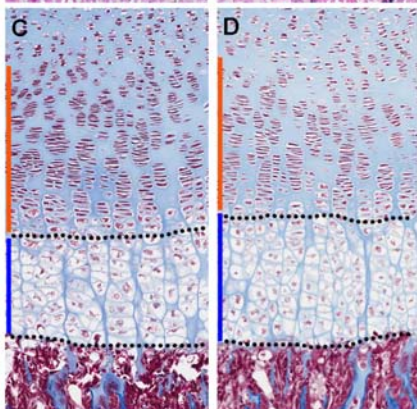


Figure S2

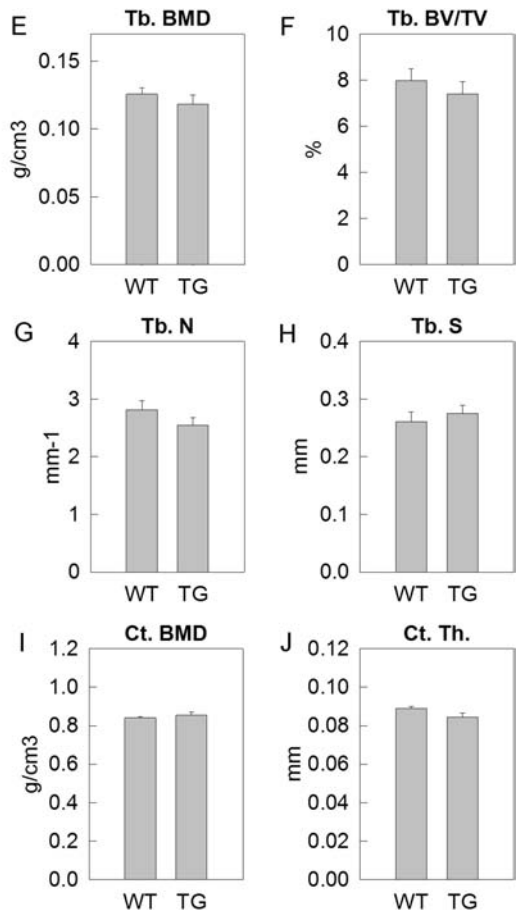
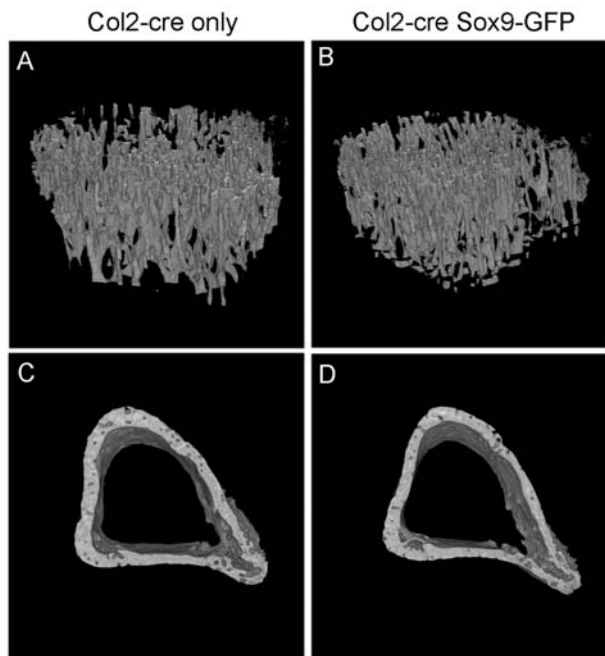


Figure S3

Supplemental Figures

Figure S1 Sox9 expression decreases in the hypertrophic zone of the murine growth plate.

(A) Laser capture microdissection (LCM) followed by qPCR showed changes in Col2, Sox9, and ColX gene expression (normalized to 18S RNA) across the different zones (RZ, resting zone; PZ, proliferative zone; PHZ, prehypertrophic zone; HZ, hypertrophic zone) of 1 wk old murine growth plates and in the adjacent trabecular bone (TB). Sox9 expression decreased dramatically in the hypertrophic zone, and continued to decrease in the trabecular bone. (Number of animals, N=5) (B-C) In situ hybridization of Collagen X (B) and Sox9 (C) provides validation of the qPCR results. (D) Masson-Trichrome-stained 1-wk old murine growth plate section, used to illustrate the areas typically dissected out by LCM for analysis. Scale bar, 100 μ m.

Figure S2. Growth plates from transgenic SOX9 mice showed slight expansion of hypertrophic zone but no effect on overall bone growth. In situ hybridization for collagen X (A,B, for 1-wk; E,F, for 4-wk), indicative of hypertrophic differentiation, showed that Col2-cre SOX9 transgenic mice have only slightly expanded areas of collagen X-expressing hypertrophic cells in both 1- and 4-wk tibial growth plates compared with Col2-cre only littermates. Masson-Trichrome-stained sections (C,D, for 1-wk; G,H, for 4-wk), showed that growth plates from Col2-cre SOX9 transgenic mice were histologically similar to Col2-cre-only littermates. Vertical bars: orange, proliferative zone; blue, hypertrophic zone. Scale bar, 100 μ m. Body weights (I) and tibia lengths (J) were compared between Col2-cre SOX9 transgenic mice and Col2-cre only littermates at 4-wk and showed no significant difference.

Figure S3. Transgenic SOX9 mice showed no significant difference in bone structure. (A-D)

3D rendering images of μ CT of tibia from 4-wk old Col2-cre SOX9 transgenic mice and Col2-cre only littermates showed similar trabecular structure and cortical thickness. (E-J) Quantitative measurements of trabecular and cortical bone with μ CT similarly showed that the tibial structure between Col2-cre SOX9 transgenic mice and Col2-cre only littermates at 4-wk were not statistically significantly different (N=7). Tb. BMD, trabecular bone mineral density; Tb. BV/TV, trabecular bone volume per tissue volume; Tb. N, trabecular number; Tb. S, trabecular spacing; Ct. BMD, cortical bone mineral density; Ct. Th, cortical thickness.