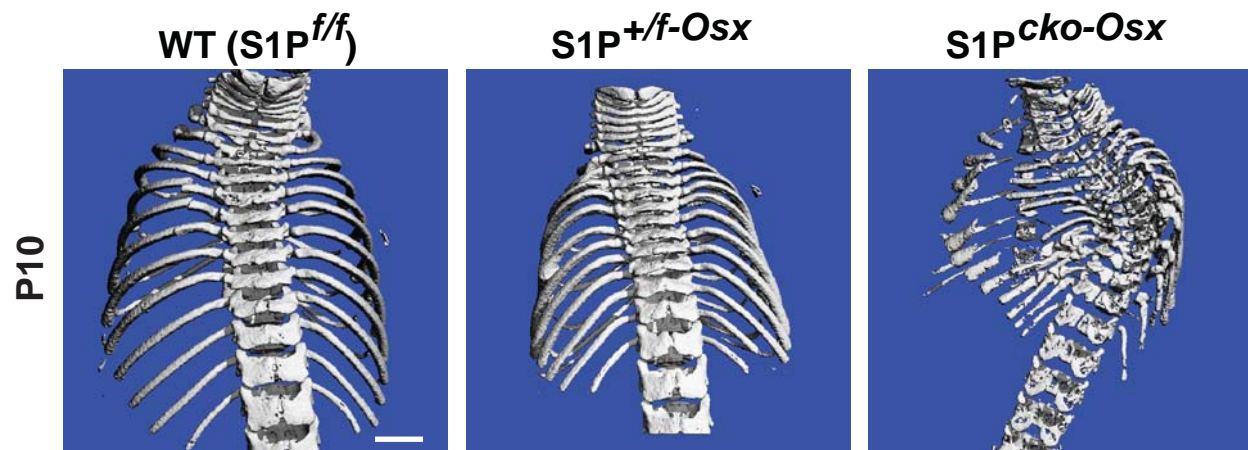


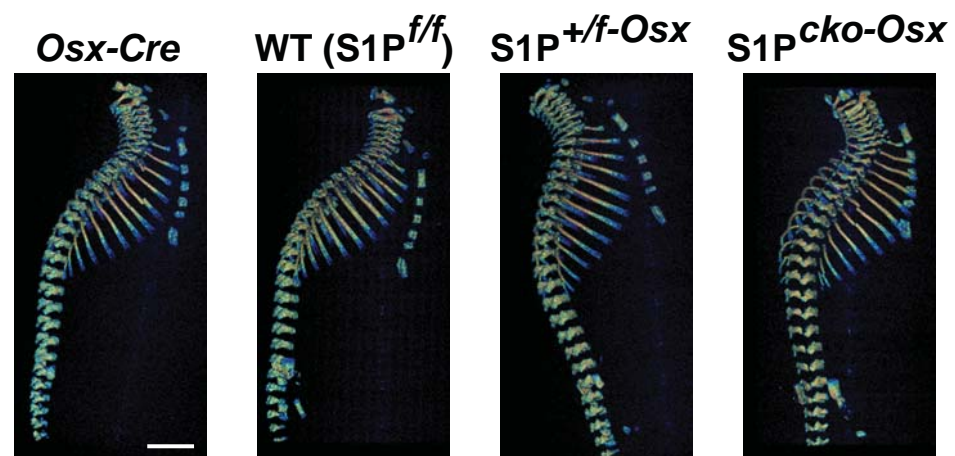
SUPPLEMENTAL MATERIALS

SUPPLEMENTAL FIGURES

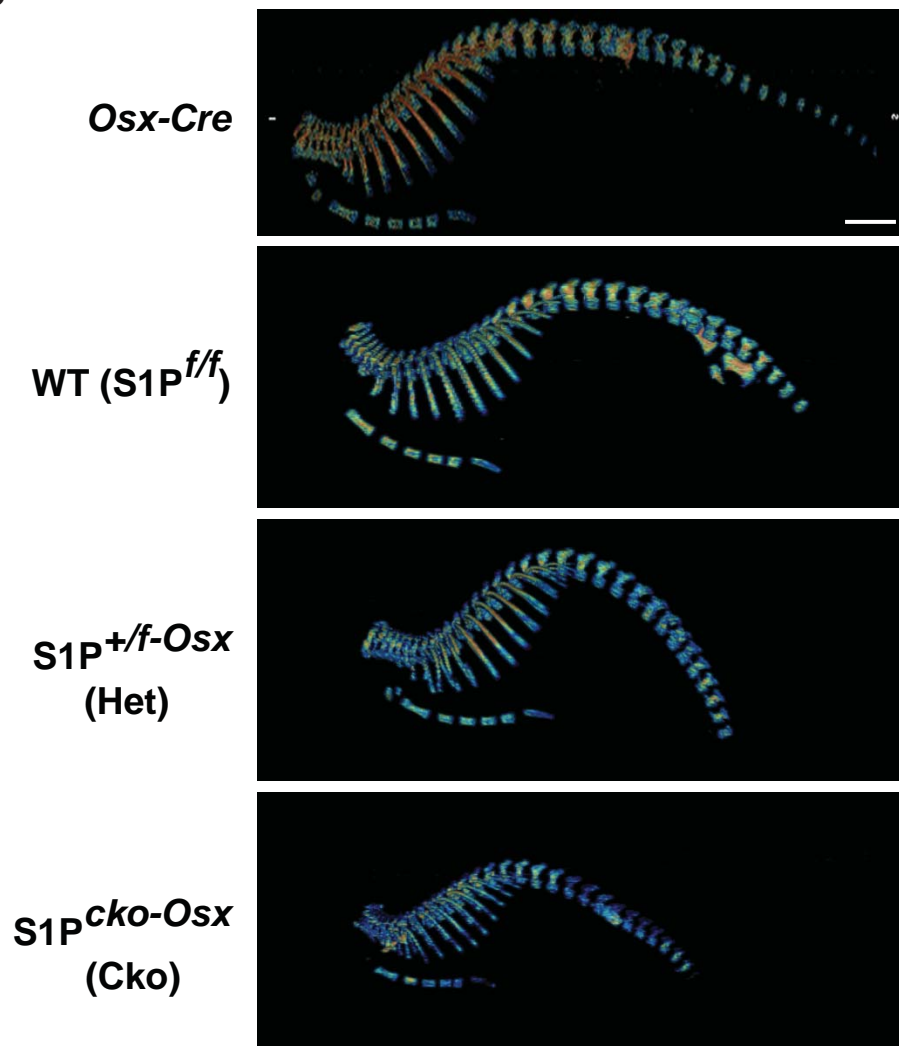


Supplemental Figure 1. Images from μ CT scans of P10 WT (S1P^{f/f}), S1P^{+/-}-O_{sx} (Het) and S1P^{cko}-O_{sx} (Cko) mice showing scoliosis in S1P^{cko}-O_{sx}. Bar: 2.5 mm.

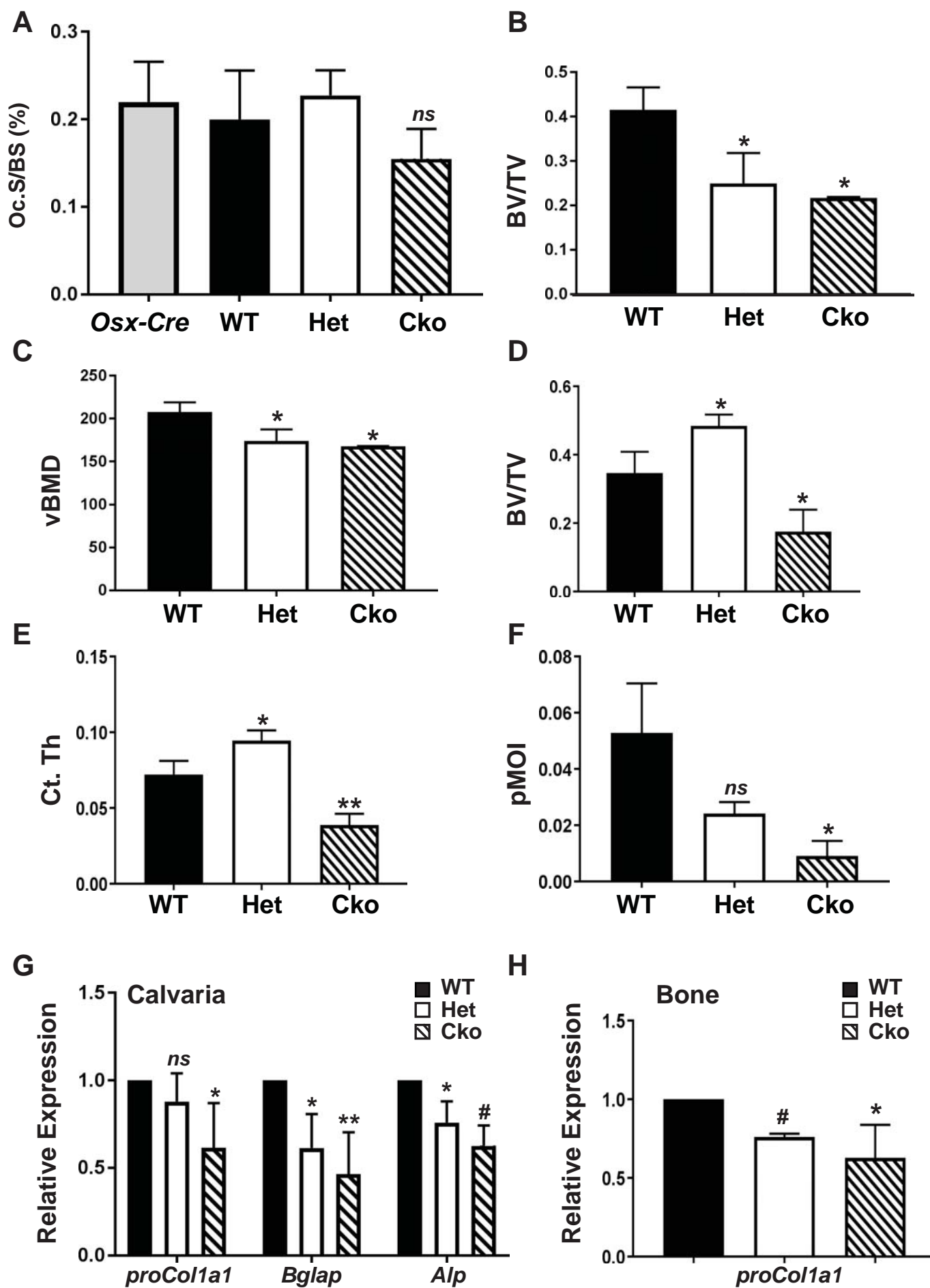
A. P1



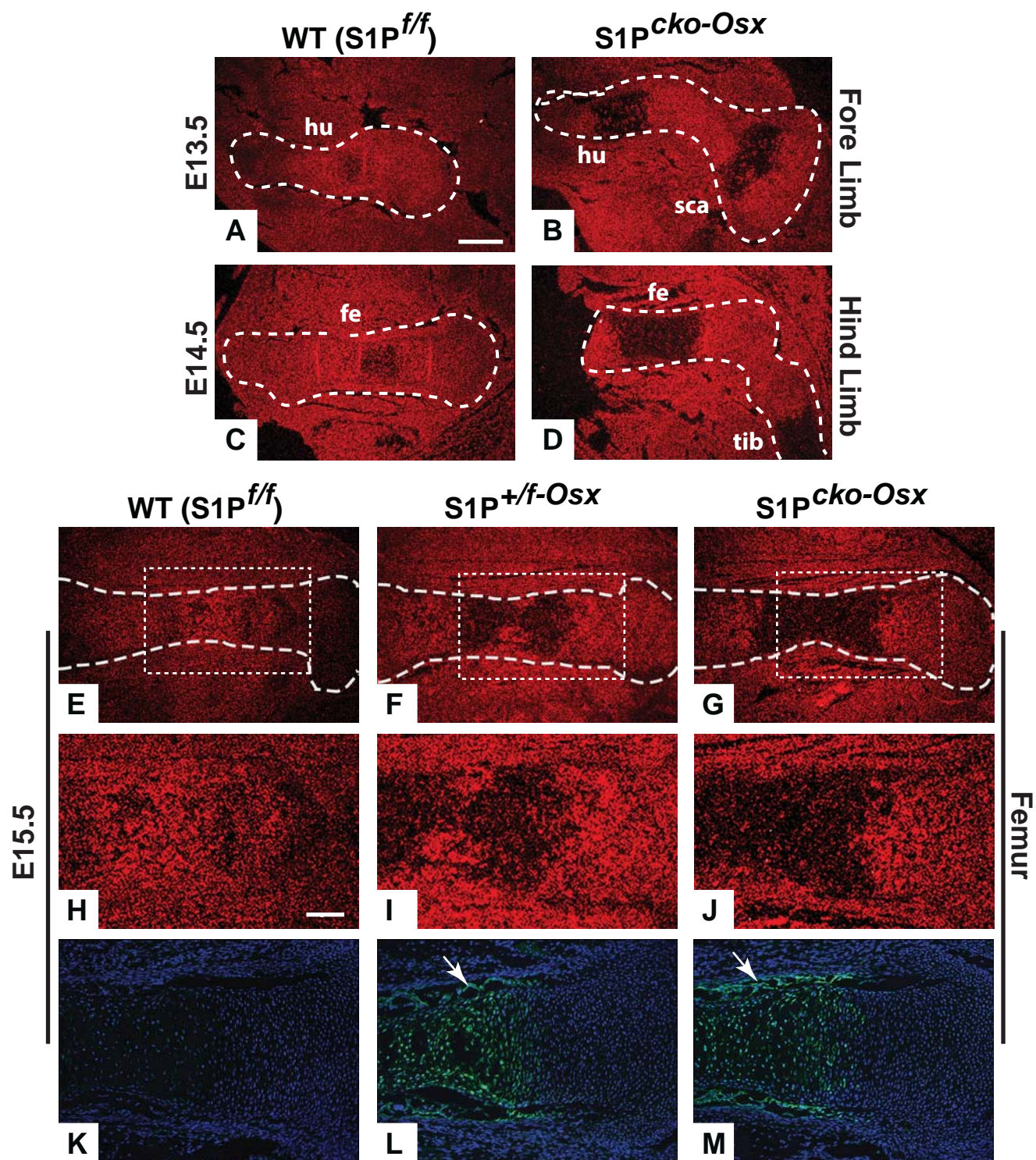
B. P5



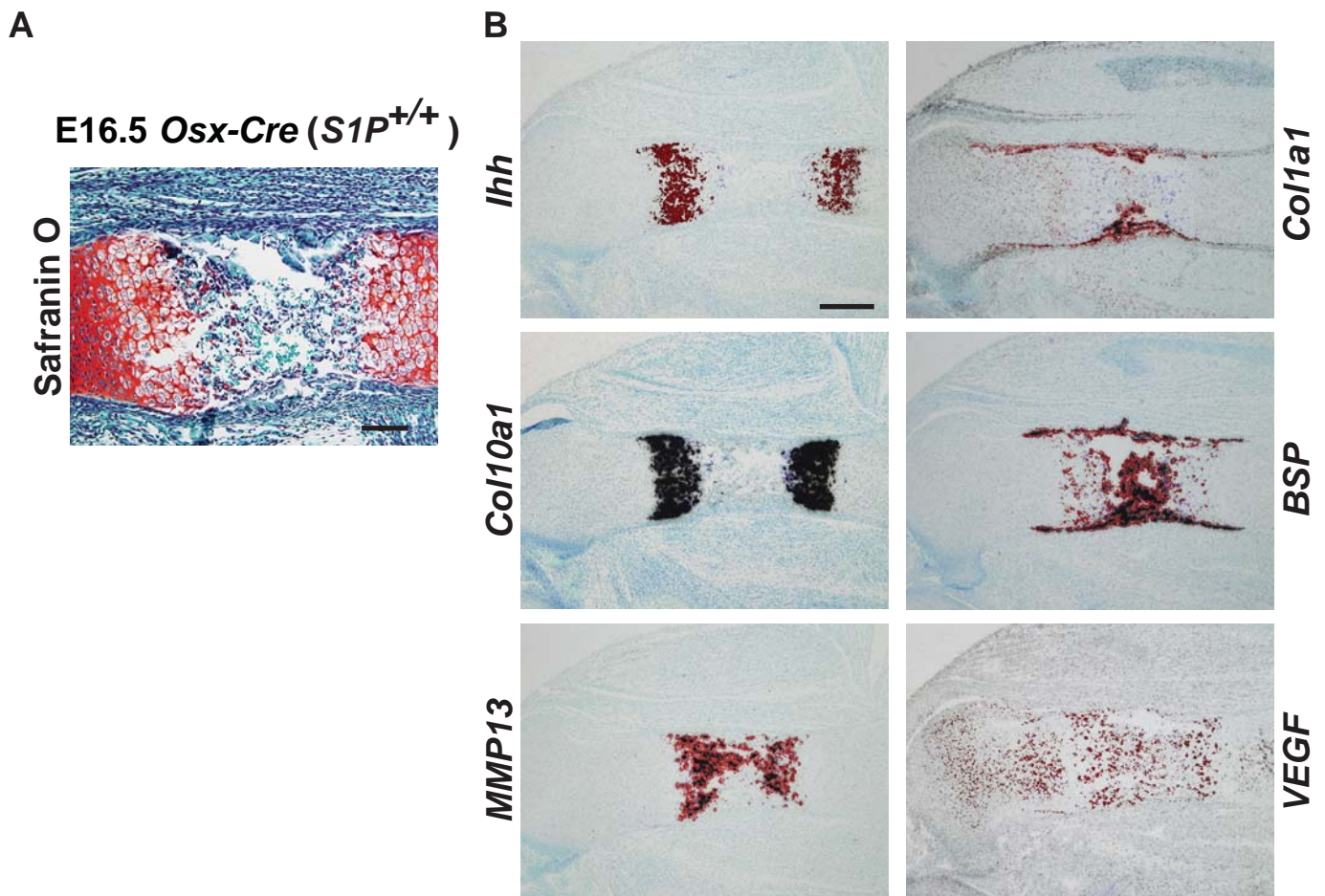
Supplemental Figure 2. Scanned μ CT images of mice axial skeletons were processed by OsiriX software and Jet color scheme using a window location of 1440 and window width of 1890 to create bone mineral density (BMD) heat maps in P1 (**A**) and P5 (**B**) *Osx-Cre*, WT (*S1P^{fl/fl}*), *S1P^{+/-Osx}* (Het) and *S1P^{cko-Osx}* (Cko) mice. A typical representation of BMD heat maps from studies of several litters is shown. Bar (A, B): 2.5 mm.



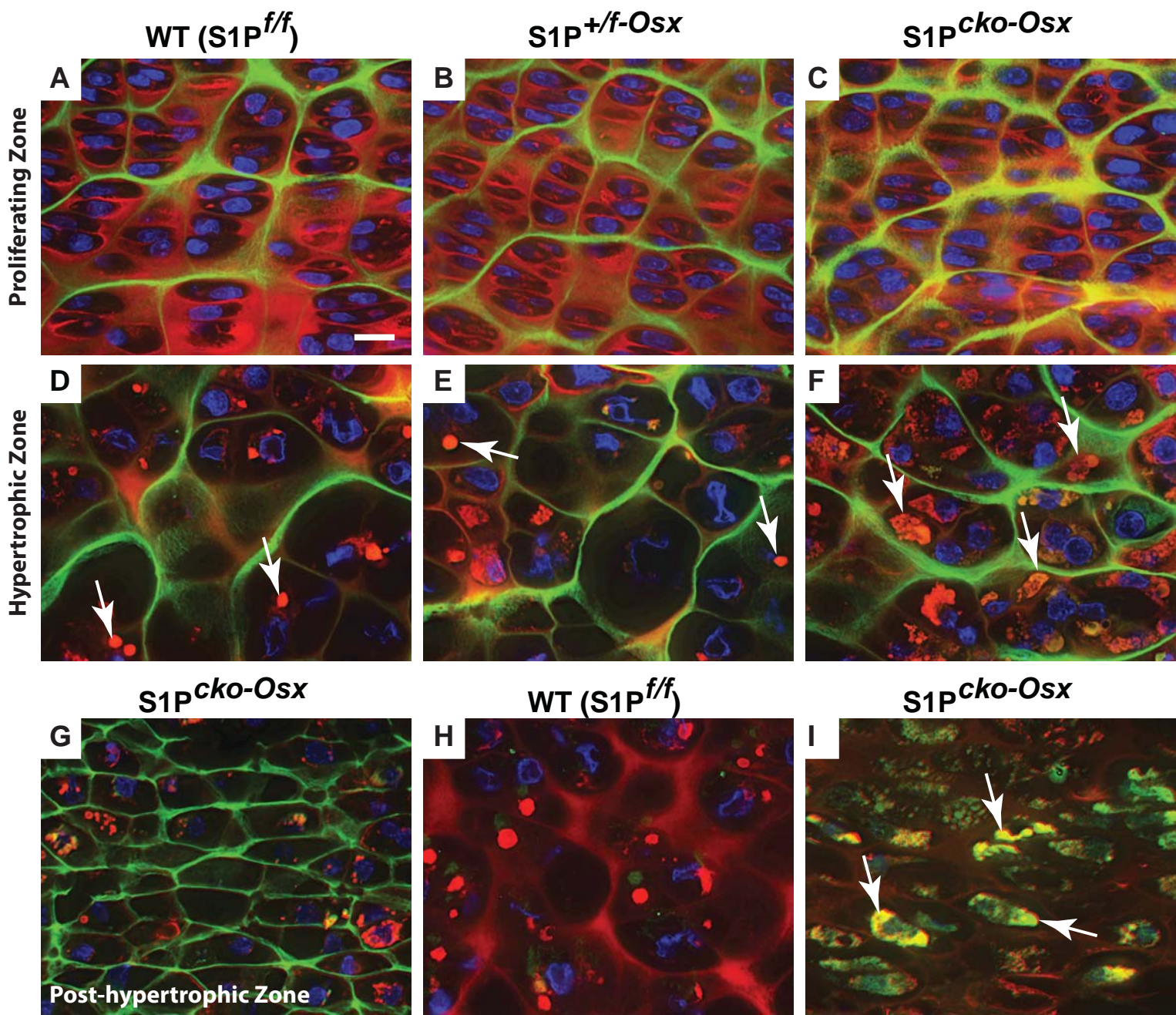
Supplemental Figure 3. (A) Dynamic histomorphometric measurement of osteoclast surface/bone surface (Oc.S/BS) (mean \pm s.d.; N=5) for P21 *Osx-Cre*, WT, Het and Cko mice ($P = ns$ (not significant)). Morphological analysis from μ CT scans for the metaphyseal trabecular bone in the distal femora (**B, C**) and mid-diaphyseal cortical bone (**D-F**) in P7 mice (mean \pm s.d.; N=3; $*P < 0.05$, $**P < 0.01$, or ns when compared to WT). (BV: bone volume; TV: tissue volume; vBMD: volumetric bone mineral density in milligrams of hydroxyapatite per cubic centimeter; Ct. Th: cortical thickness; pMOI: polar moment of inertia as a measure of resistance to torsional force). Q-PCR analysis for *proColla1*, *Bglap*, and *Alp* with RNA harvested from P21 calvaria (**G**) and long bones (femur/tibia with bone marrow flushed out) (**H**) (mean \pm s.d.; N=3, $*P < 0.05$, $**P < 0.01$, $^{\#}P < 0.005$ or ns , when compared to WT).



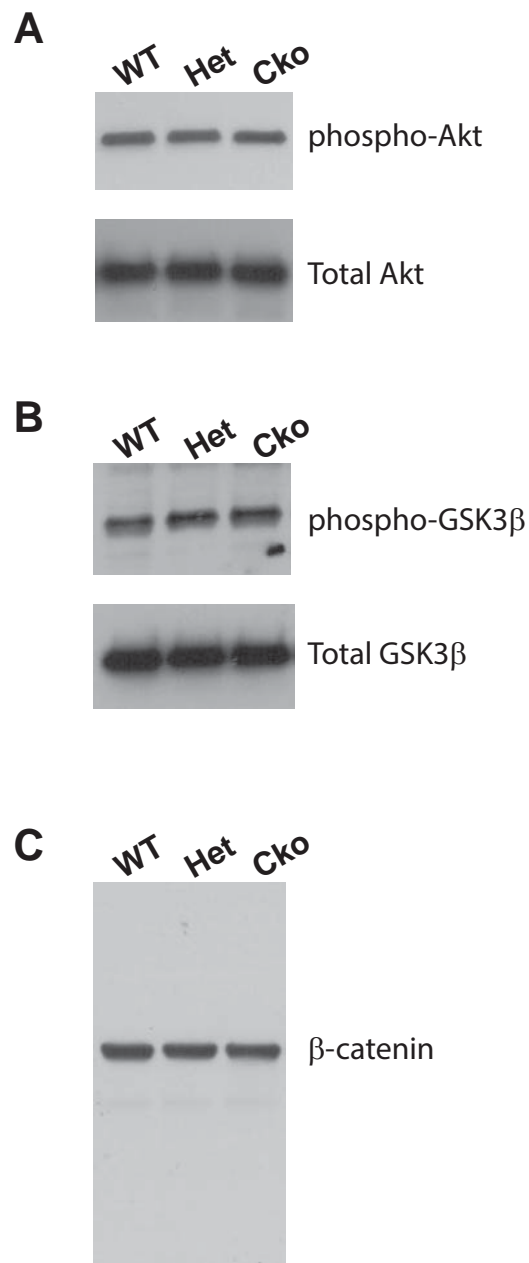
Supplemental Figure 4. (A-G) *In situ* hybridization analysis for *Mbtps1-exon 2* at E13.5 (A and B, in fore limb) and E14.5 (C and D, in hind limb) in WT and S1P^{cko-Osx} mice, and at E15.5 in WT, S1P^{+/-Osx} and S1P^{cko-Osx} mice (E-G, femur). Signals from *Mbtps1-exon 2* are shown in red. Notice the loss of expression in the pre-hypertrophic/hypertrophic zones in the growth plate (hu: humerus; sca: scapula; fe: femur; tib: tibia). Panels H-J are higher magnification images of panels E-G of the region demarcated by the dashed rectangle and are shown in the same magnification as panels K-M. (K-M) GFP expression (due to expression of the GFP-Cre fusion protein from the *Sp7* (*Osx*) promoter) in E15.5 femora in S1P^{+/-Osx} (Het) and S1P^{cko-Osx} (Cko) mice, absent in WT, showing the tissue distribution of *Osx-Cre* activity and its overlap with *Mbtps1-exon2* ablation (E-J). Arrow points to GFP expression in the perichondrium indicating *Mbtps1-exon 2* deletion in perichondrial bone progenitors. Bar (A-G): 250 μ m; (H-M): 100 μ m.



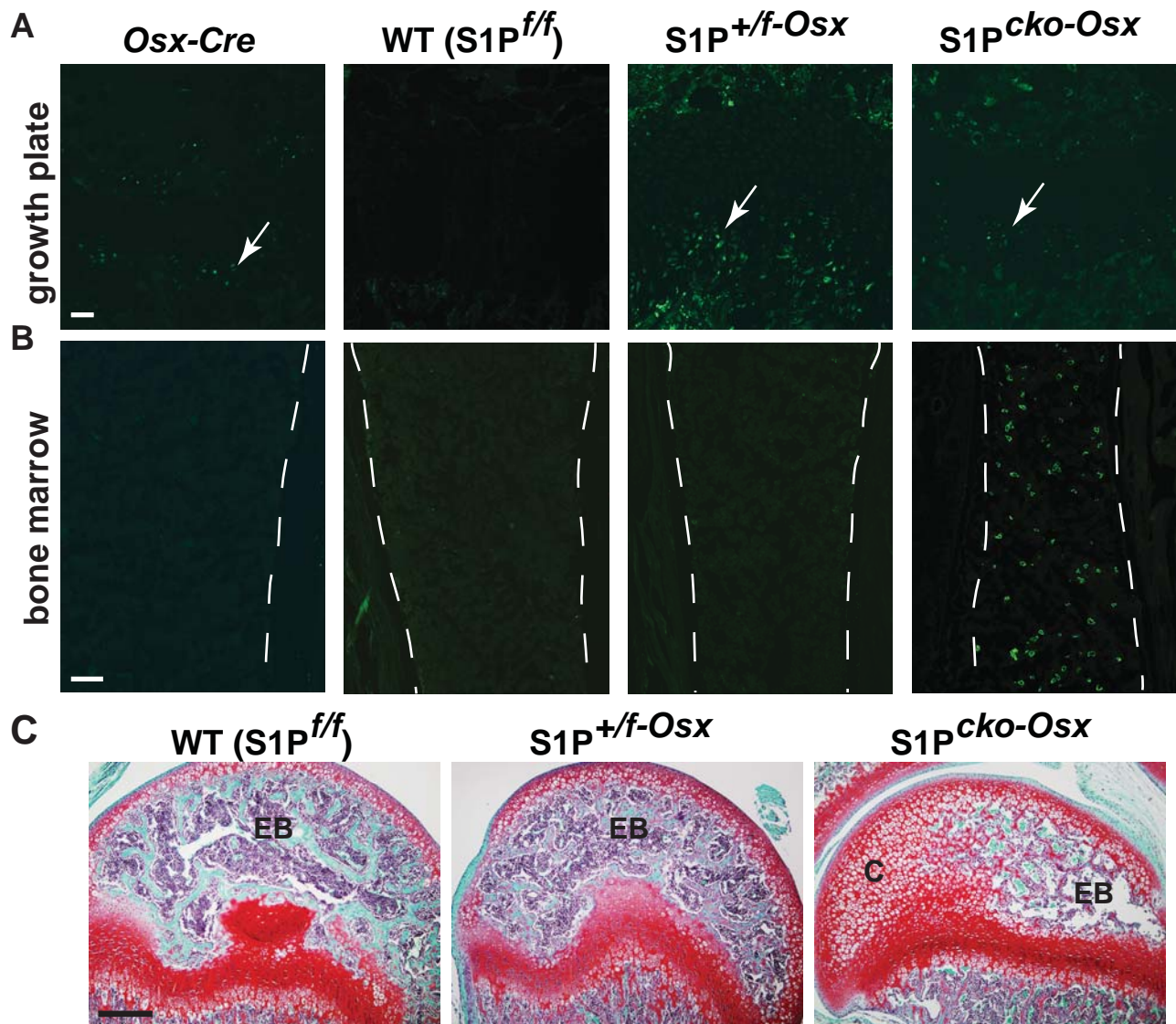
Supplemental Figure 5. (A) Safranin O, Fast green, and hematoxylin stained femur from a control *Osx-Cre* (*S1P*^{+/+}) mouse at E16.5. Bar: 100 μ m. (B) *In situ* hybridization analyses in *Osx-Cre* control mouse in E16.5 femur for *Ihh*, *Col10a1*, *MMP13*, *Col1a1*, *BSP*, and *VEGF*. Bar (all panels): 250 μ m.



Supplemental Figure 6. Entrapment of pro-Col IIB in the pre-hypertrophic/hypertrophic chondrocytes in $S1P^{cko-Osx}$. (**A-G**) Double-labeled immunofluorescence for Col II THD (red) and Col IIA (green) in E16.5 femora in WT (**A, D**), $S1P^{+/f-Osx}$ (**B, E**) and $S1P^{cko-Osx}$ (**C, F**) are shown for proliferating cells (**A-C**), pre-hypertrophic/hypertrophic chondrocytes (**D-F**), and the post-hypertrophic zone (**G**, $S1P^{cko-Osx}$ only). Arrows point to Col II THD clumps in WT (**D**) and $S1P^{+/f-Osx}$ (**E**) hypertrophic matrix, or the intracellularly trapped Col II in $S1P^{cko-Osx}$ (**F**). (**H, I**) Double-labeled immunofluorescence for pro-Col IIB (green) and Col II THD (red) in E16.5 femora in WT (**H**) and $S1P^{cko-Osx}$ (**I**). Arrows (**I**) point to yellow colocalization signals from pro-Col IIB and THD indicating pro-Col IIB entrapment. Bar (all panels): 10 μ m.



Supplemental Figure 7. Immunoblot analysis of Wnt/ β -catenin signaling components in embryonic bone. E14.5 forelimbs were harvested from WT, $S1P^{+/f-Osx}$ (Het) and $S1P^{cko-Osx}$ (Cko) mice, proteins extracted and equal amounts of limb lysates loaded and analyzed by western blot with the antibodies indicated.



Supplemental Figure 8. Distribution of GFP⁺ cells in the growth plate (A) and bone marrow (B) in P21 *Osx-Cre*, WT (*S1P^{f/f}*), *S1P^{+/-}-Osx* (Het) and *S1P^{cko}-Osx* (Cko) mice. Dashed lines show the junction of bone marrow with cortical bone. Arrows point to the location of GFP⁺ cells in the pre-hypertrophic/hypertrophic zone of the growth plates. (C) Safranin O stained sections from P21 mice showing the secondary ossification center in the proximal humerus. Notice the smaller size of the humeral head in *S1P*-ablated mice and the incomplete removal of cartilage in *S1P^{cko}-Osx* (C: cartilage; EB: epiphyseal bone). Bar (A): 50 μ m; (B): 100 μ m; (C): 250 μ m.