

Supporting Information

Regan et al. 10.1073/pnas.1324290111

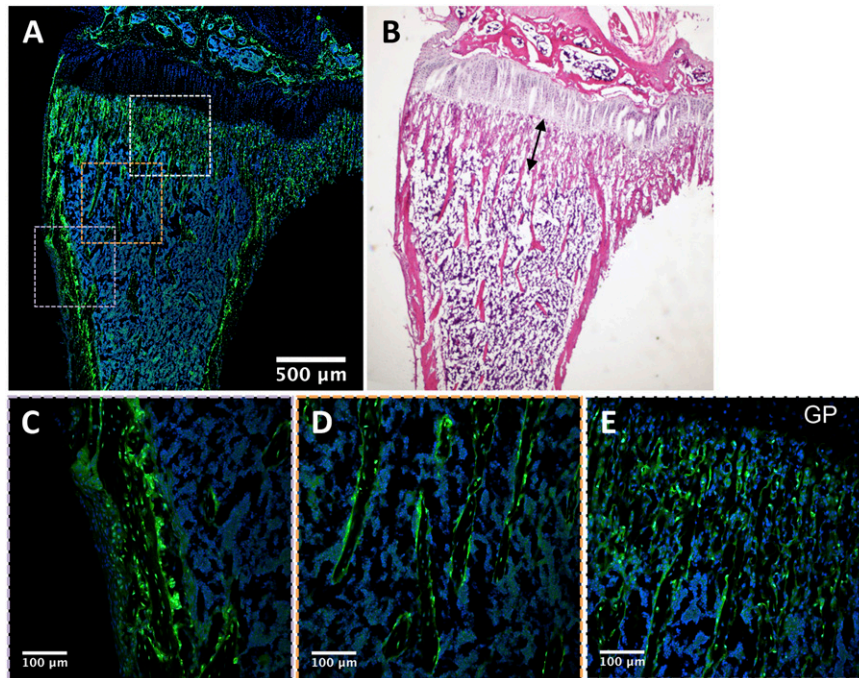


Fig. S1. SP7-tTA marks osteoblast-lineage cells. (A) Cryosection of the tibia following 15 d of doxycycline (dox) removal. GFP-positive cells indicate SP7 transgene expression. Areas of interest are boxed and shown at a higher magnification in C–E. (B) H&E staining of the same section shown in A for anatomical orientation. Double-headed arrow denotes the chondro–osseous junction rich in osteoblast precursors. (C) Cortical bone. (D) Cancellous bone. (E) Chondro–osseous junction region containing osteoblast precursors. GP, growth plate.

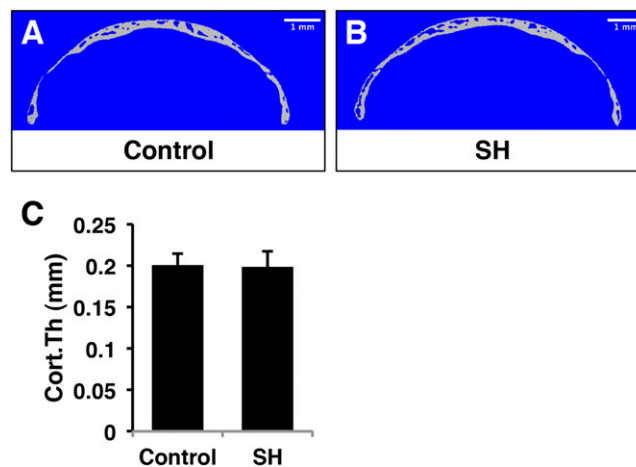


Fig. S2. Calvarium and cortical bone are not affected by HIF1-PPN expression during the 15-d experimental window. (A and B) Comparison of anatomically equivalent sections of calvarium from control (A) and SH (B) mice. μ CT scans of parietal bone are shown. (C) Quantification of cortical thickness taken from μ CT scans of 1.6 mm of tibia midshaft immediately proximal to the intersection of tibia and fibula ($n = 3$).

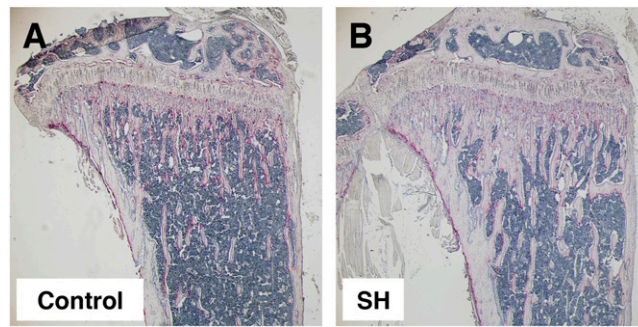


Fig. S3. Tartrate-resistant acid phosphatase (TRAP) staining for osteoclasts. (A and B) Low-magnification views of control (A) and SH (B) tibia sections with TRAP staining.

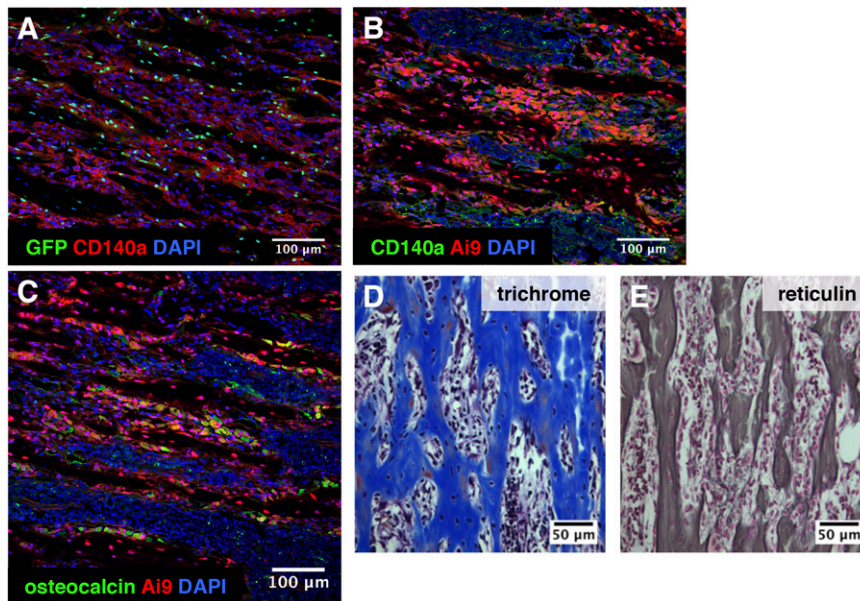


Fig. S4. Characterization of intertrabecular fibrosis in SH mice. (A) Immunofluorescent (IF) staining for GFP (green; SP7-positive cells), CD140a (red; generic mesenchymal cell marker), and DAPI (blue; nuclei). Fibrotic cells between trabeculae (which appear as black areas with few nuclei) are CD140a-positive but mostly negative for GFP. (B) IF staining for CD140a (green), Ai9 (red; lineage tracing of any cells that have expressed SP7-Cre::GFP during the 15-d experimental window), and DAPI (blue). A subset of CD140a-positive intertrabecular fibrotic cells is also Ai9-positive. (C) IF staining for osteocalcin (green), Ai9 (red), and DAPI (blue). (D) Masson's trichrome staining of collagen I (blue) in tibia. Little staining is seen in the fibrotic areas between the trabeculae, which are bright blue. (E) Reticulin staining for type III collagen (black) in tibia. Thin black fibrils are observed interspersed with the red nuclei in the fibrotic regions between the trabeculae.