Supporting Information

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SI Materials and Methods

Quantifying Transcript Levels. Tissues were dissected in ice-cold PBS, and bones were flushed free of marrow and dissected free of adherent tissue. Bones were crushed in QIAzol or RLT buffer with 2-mercaptoethanol (Qiagen) then homogenized using a rotor-stator homogenizer, from which RNA was purified using RNeasy kit (Qiagen). RNA quantification and purity was tested by measuring the absorbance at 230, 260, and 280 nm. RNA quality was checked on a 2% agarose gel for the presence of 18S and 24S ribosomal RNA bands. One microgram of RNA was reverse-transcribed with QuantiTect Reverse Transcription kit (Qiagen), which includes genomic DNA elimination. qPCR was performed with primer-probe sets (Applied Biosystems) for *Mef2C*, *Sost*, *Opg*, *Rankl*, and *Rpl32* using QuantiFast Probe PCR kit (Qiagen). Cycling conditions were 95 °C for 3 min, followed by 40 cycles of 95 °C for 3 s, and 30 s at 60 °C. qPCR results were calculated relative to internal control (*Rpl32*; $2^{-\Delta Ct}$).

Serum Immunoassays. Mouse serum was collected at 6 mo of age and diluted 1:1 with diluent provided by the manufacturer. All assays were performed in duplicate or triplicate against a set of standards provided by the manufacturer [CTX-1 (RatLaps EIA ImmunoDiagnostic Systems, cat. AC-06F1); RANKL (R&D Systems, cat. MTR00). WT, *Mef2C*^{cKO}*;Col1-Cre*, and *SOST*^{KO} or *ECR5*^{KO} were compared. Values were fit to standard curves and the concentration of antigen in serum was extrapolated. Groups were compared using unpaired Student's *t* test.

LacZ, GFP, Immunofluorescence/Immunohistochemistry, and Histology. LacZ staining was performed as described (35). GFP expression was determined by whole-mount photography of freshly dissected tissues. Bones from adult mice were dissected, decalcified and embedded in paraffin. Blocks were sectioned at 6 μm, air-dried for 2–4 h, and baked overnight at 42 °C. Slides were dewaxed, and epitopes requiring antigen retrieval were incubated in Uni-Trieve (Innovex) for 30 min at 65 °C. Slides were blocked with 5% BSA/0.01% Triton X-100 (vol/vol) (Sigma) or Rodent Block (Innovex), incubated in a humid chamber with primary antibody overnight at room temperature [anti-Sclerostin (R&D Systems), anti-Mef2C (Abcam), anti-Periostin (Abcam), anti-GFP (Abcam); anti-activated-β-catenin (Millipore)], washed, and incubated for 2 h with Alexa-fluor-labeled secondary antibody (1:1,000, Invitrogen/Molecular Probes) or HRP-conjugated secondary antibody and DAB (1:1,000, Jackson ImmunoResearch, anti-Sclerostin only). Slides were then washed and mounted using Prolong Gold/Prolog Gold with DAPI (Invitrogen/Molecular Probes) or Permount for imaging. Images were acquired using single-channel fluorescent filters or brightfield on a Leica DM5000 compound microscope using ImagePro software.

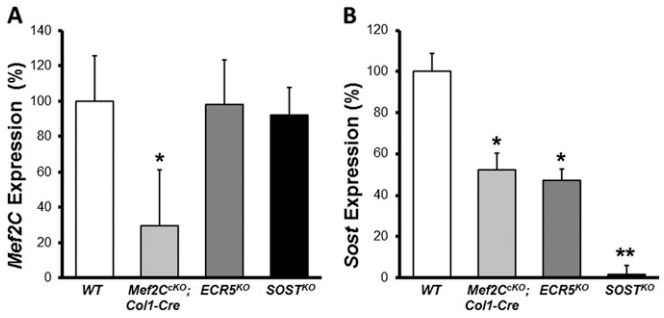

Fig. S1. Quantitative analysis of *Mef2C* (*A*) and *Sost* (*B*) transcript levels in the cortical bone of *Mef2C*^{cKO}; *Col1*-Cre, *ECR5*^{KO}, and *Sost*^{KO} mice. *Mef2C* expression was significantly reduced by ~70% in adult femurs (*A*) of *Mef2C*^{cKO}*;Col1*-Cre mice relative to WT controls. No significant *Mef2C* expression differences were observed in *ECR5*^{KO} and *Sost*^{KO} relative to WT femurs. *Sost* transcript levels were reduced by ~50% in the femurs (*B*) of both *ECR5*^{KO} and *Mef2C*^{cKO}*;Col1*-Cre mice relative to WT controls; *Sost* expression was absent in *Sost*^{KO} mice. Values shown are relative mean percentage ± SEM, WT represented at 100% relative expression; $n = 3$–6 per group; $^{*}P < 0.05$; $^{**}P < 0.01$.

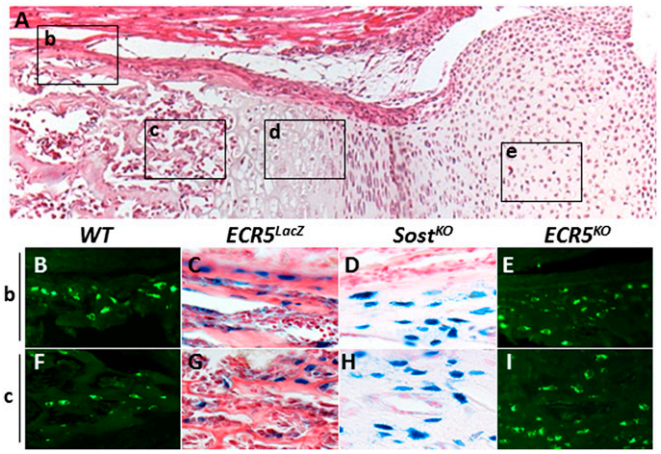

Fig. S2. Comparing Sost bone Expression in WT, *ECR5*^{LacZ}, *Sost*^{LacZ}, and *ECR5*^{KO} mice. We examined *Sost* and *LacZ* reporter expression in WT, *ECR5*^{LacZ}, *Sost*^{LacZ}, and *ECR5*^{KO} mice in neonatal long bones. (*A*) Four regions were examined: periosteum/cortical bone (b), trabecular (c), hypertrophic chondrocyte (d) and proliferating chondrocyte (e) regions were examined. We found *Sost* to be expressed only in osteocytes in the periosteum and cortical bone (*B*–*E*) and trabeculae (*F*–*I*); no Sost expression was detected in hypertrophic (d) or proliferating (e) chondrocyte regions. Sost expression was highly variable in *ECR5*^{KO}; here, we show regions that maintained Sost expression (*E* and *I*), but most osteocytes lacked Sost expression, as shown in Fig. 4*L*.

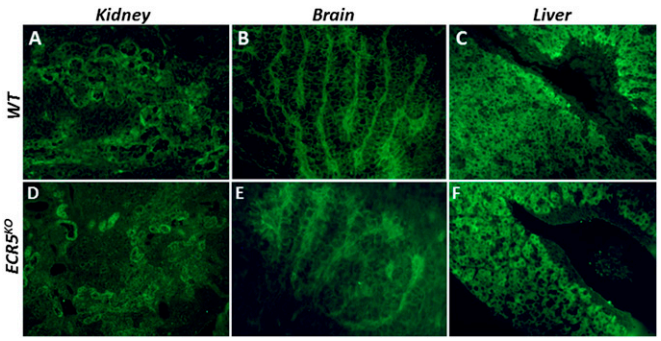

Fig. S3. Sost expression in kidney, brain, and liver was unaffected in *ECR5*^{KO} mice. Sost protein levels were examined by fluorescent immunohistochemistry in the kidney (*A* and *D*), brain (*B* and *E*), and liver (*D* and *F*), and no significant differences between WT (*A*–*C*) and *ECR5*^{KO} (*D*–*F*) were observed.

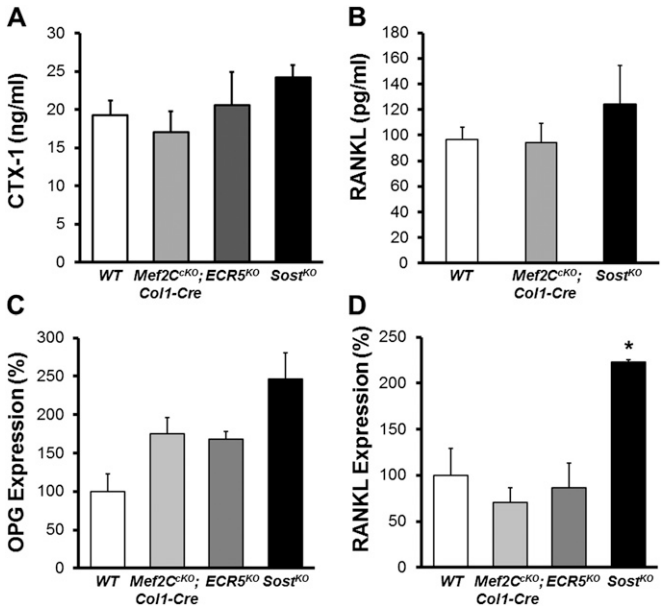

Fig. S4. Examining markers of osteoclast activity. Immunoassays quantifying CTX-1 (*A*) and RANKL (*B*) serum protein levels were carried out on samples derived from the same animals examined by μCT/histomorphometry. No significant differences were observed among the four groups (WT, *Mef2C^{cKO};Col1-Cre*; *ECR5^{KO}*; *Sost^{KO}*). *Opg* (*C*) and *Rankl* (*D*) transcript levels were also quantified in cortical bone. *Opg* expression, though slightly elevated above WT levels in all HBM groups, did not reach statistical significance in any group relative to WT (*C*). *Rankl* expression was found to be significantly up-regulated in *SOST^{KO}* only, with no significant differences among all other groups (*D*). OPG and RANKL values are shown as relative mean percentage ± SEM, WT is represented at 100% relative expression; n = 3–6 per group; $^*P < 0.05$.

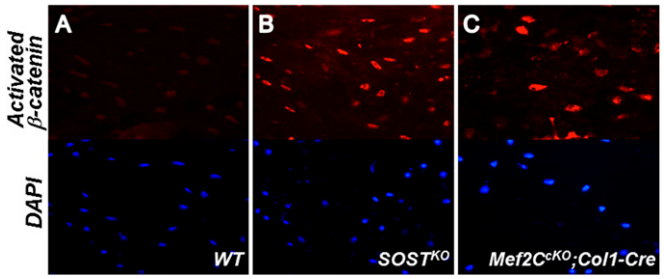

Fig. S5. β-Catenin activation is increased in HBM. Activated β-catenin protein (*A*–*C*; *Upper*; red stain) was examined in cortical osteocytes of WT (*A*), *SOST^{KO}* (*B*), *Mef2C^{cKO};Col1-Cre* (*C*) mice. Same sections were counterstained with DAPI to indicate cell locations (*A*–*C*; *Lower*; blue).