Material and Methods

RNA extraction and Purification

Briefly, femurs were dissected and cleaned of adherent tissues. Distal and proximal epiphyses were removed and the bone marrow was extensively flushed out with α-Minimum Essential Medium (αMEM, Invitrogen Carlsbad, CA, USA) supplemented with 0.1 % bovine serum albumin (BSA), 25mM Hepes pH 7.4 and containing 1mg/ml of collagenase (type I:II, ratio 1:3). Bones were cut into a few pieces and sequentially digested in 0.5 ml of α-MEM containing 0.1%BSA, 1mM $CaCl_2$ and 1mg/ml of collagenase (type I and II; ratio 1:3) (Worthington Biochemical Corporation, Freehold, NJ, USA). Bones were digested 6 times for 15 min each at 37°C on a rocking platform at 90 oscillations per minute under 5% CO_2 in air. Digestions 4 and 6 were carried out in 0.5 ml of 50mM EDTA in phosphate buffer solution (PBS) containing 0.1 % BSA.

Isolation of primary bone cells from murine calvaria

Pups were injected with 50μg/gr of tamoxifen at day 2 and 4. Parietal calvarial bones were dissected from 5 day-old pups and subjected to 6 collagenase digestions as previously described (21). Briefly, calvarial bones were dissected aseptically and sequentially digested for 6 times in: a) 0.5 ml of α-Minimum Essential Medium (αMEM) containing 0.1% bovine serum albumin (BSA), 1mM $CaCl_2$ and 1mg/ml of collagenase (type I and II; ratio 1:3) (Worthington Biochemical Corporation, Freehold, NJ, U.S.A.). Bones were digested for 15 min each at 37 ^{o}C on a rocking platform at 90 oscillations per minute under 5% CO_2 in air. Cells from fractions 1 and 2 were routinely discarded. Cells from fractions 3-6 were pooled together for culture and subsequent cAMP accumulation. Cells were plated in 24-well plate and cultured until confluen. Cells were rinsed twice with assay buffer (135 mM NaCl, 6 mM KCl, 1 mM $MgCl_2$, 2.8 mM glucose, 1.2 mM $CaCl_2$, and 20 mM HEPES, pH 7.4) and then incubated for 15 min at 37 ^{o} C with the same buffer containing 0.1% heat inactivated BSA, 1 mM isobutylmethylxanthine (IBMX), and agonist, conditions under which cAMP accumulation was found to be linear with time for at least 15 min. The buffer then was rapidly aspirated, the plates were frozen in liquid nitrogen, and the frozen cells subsequently were thawed directly into 0.5 ml of 50 mM HCl. Cell-associated cAMP in the acid extracts was measured using an RIA kit (NEN Life Science

Products, Boston, MA, U.S.A.). Results were expressed as picomoles of cAMP produced per well over 15 min.

Quantitative RT-PCR.

Quantitative RT-PCR was performed using the following primer sets;

PPR-E1: 5'-GGAGTCAGACAAAGGGTGGA-3' ; PPR-E1-2 : 5'- GTGGGCACATCCTTG TTCTC; GAPDH-P1: 5'- TGGAGTCTACTGGTGTCTTCA; GAPDH-P2: 5'-AAGCAG TTGGTGGTGCAGGAT-3' ; SOST-P1: 5'-GGTGGCCTCGTGCAAGTGCAA-3' ; SOST-P2: 5'-TAGGCGTTCTCCAGCTCCG-3'; AXIN2-P1: 5'-ATGTGTGGATACGCTGGACTT-3', AXIN2-P2 5'-TTCTTGATGCCATCTCGTATG-3'.

Supplemental Figures

Figure 1S: C) Southern Blot analysis of Cre-recombinase positive mice. Genomic tail DNA from a positive F1 (lane2) and her progeny (F2 : P1 to P8) was digested with *EcoR I* (unique site) and probed for Cre-recombinase gene. A Cre-specific probe was cloned using PCR primers (shown in the diagram). Southern Blot analysis confirmed germline transmission and single site of insertion. Marker shown on the left: Lambda DNA/*Hind III.*

Figure 2S: X-gal staining of kidney of ROSA26;DMP1-Cre positive (A) and negative (B) animal. 20X magnification. C) X-gal staining of skeletal muscle and cortical bone of tibia of DMP1-Cre positive animal. Note lack of staining in skeletal muscle 20X magnification. D) X-gal staining of femur from DMP1-Cre positive animals. Note lack of blue staining in bone marrow cells. 10X magnification.