Supporting Information

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SI Text

In Vivo Parathyroid Hormone Experiment. ***Generation of*** **Postn$^{-/-}$** ***mice.*** *Periostin* (*Postn*)$^{-Lac\ Z}$ knock-in mice (*Postn*$^{-/-}$) were generated as reported previously (1). *Postn*$^{-/-}$ mice were subsequently bred with C57BL/6J mice, and ear DNA analyzed by PCR was used to identify *Postn* heterozygous mice. We interbred mice that were heterozygous carriers of this mutation and obtained wild-type (*Postn*$^{+/+}$) and homozygous mutant (*Postn*$^{-/-}$) offspring in the expected Mendelian genetic frequencies. These mice were subsequently back-crossed for six generations, resulting in a genome of 98% C57BL/6J. Mice were housed five per cage, maintained under standard nonbarrier conditions, and had access to water and soft diet ad libitum (Harlan Teklad 2019, SDS). A soft diet was chosen to reduce the severity of periodontal disease, which was previously observed in the *Postn*$^{-/-}$ mice under a standard diet (1). All of the mice received the same diet throughout the experiment. Twelve-week-old *Postn*$^{-/-}$ and *Postn*$^{+/+}$ female mice were treated with either daily subcutaneous parathyroid hormone (PTH) (40 μg·kg·d) or vehicle for 5 wk. To measure dynamic indices of bone formation, mice were injected with calcein 9 and 2 d before being killed. The animals were killed and blood was collected for serum measurements. Lumbar spine and femurs were excised for microcomputed tomography analysis, histomorphometry, and biomechanical analysis.

This experiment was repeated in *Postn*$^{+/+}$ and *Postn*$^{-/-}$ mice with concomitant intravenous injection of a sclerostin-blocking antibody (Sost-Ab, 12 mg·kg·wk) or a control antibody (anticyclosporin A, Ig-G2A) for 5 wk (n = 8 mice per group per genotype). The anti-Sost antibody was isolated from a combinatorial antibody library using phage display technology (MorphoSys) and administered 1 h before intermittent PTH (iPTH) at a concentration previously found to have mild anabolic effects on bone (2). Animal procedures were approved by the University Of Geneva School Of Medicine Ethical Committee and the State of Geneva Veterinarian Office.

Generation of **Postn$^{-/-}$** ***TOPGAL mice.*** We used the Wnt indicator TOPGAL mice to identify if periostin could have a role in this pathway activated by PTH. TOPGAL mice were breed with *Postn*$^{-/-}$ to generate *TOPGAL Postn*$^{-/-}$ and *TOPGAL Postn*$^{+/+}$ mice. The mice were treated with either daily subcutaneous PTH (40 μg·kg·d) or vehicle for 1 wk (n = 3 mice per group per genotype). β-Galactosidase in adult bone was detected as previously described by Hens et al. (3). In brief, adult bone were fixed in 2% (vol/vol) paraformaldehyde and 0.02% glutaraldehyde in PBS for 1 h at room temperature and washed twice in PBS. Bones were first decalcified in 15% EDTA for 1 mo and then washed in PBS for 3 h before being incubated in 0.1% 4-chloro-5-bromo-3-indolyl β-D-galactosidase (X-Gal solution; Sigma) at 37 °C overnight. Bones were subsequently washed once with PBS and then postfixed in 4% paraformaldehyde at 4 °C overnight. Individual bones were rinsed in 70% ethanol, embedded in paraffin way, sectioned, and counterstained with eosin. Primary osteoblast cultures isolated from *TOPGAL Postn*$^{+/+}$ and *Postn*$^{-/-}$ newborn calvaria were exposed to PTH (10^{-7} M) or recombinante periostin (2 μg/mL) for 24 h at 14 d of culture (see below for calvaria culture detail). To detect β-galactosidase in primary calvaria osteoblasts from TOPGAL mice, cells were washed once in PBS, fixed for 10 min in 2% paraformaldehyde, washed twice with PBS, and stained overnight in X-Gal solution at 37 °C (Sigma). After this process, cells were washed twice with PBS and fixed in alcohol for observation. To evaluate β-catenin expression, cells were digested in collagenase and detached from coated plates by trypsin. Cells were centrifuged 5 min at 3,000 × g and the pellets were dissolved in 1 mL of αMEM. Next, 10 μL αMEM was inserted in the Neubauer chamber. Each experiment was performed in triplicate.

Ex vivo measurement of bone microarchitecture. Microcomputed tomography (microCT UCT40; Scanco Medical) was used to assess trabecular bone volume fraction and microarchitecture in the excised fifth lumbar spine body and distal femur, and cortical bone geometry at the midshaft femoral diaphysis, as previously described (4). Briefly, trabecular and cortical bone regions were evaluated using isotropic 12-μm voxels. For the vertebral trabecular region, we evaluated 250 transverse CT slices between the cranial and caudal end plates, excluding 100 μm near each endplate. For the femoral and tibial trabecular region, to eliminate the primary spongious, we analyzed one hundred slices from the 50 slices under the distal growth plate. Femoral cortical geometry was assessed using 50 continuous CT slides (600 μm) located at the femoral midshaft. Images were segmented using an adaptative-iterative thresholding approach rather than a fixed threshold. Morphometric variables were computed from binarized images using direct, 3D techniques that do not rely on prior assumptions about the underlying structure (5). For the trabecular bone regions, we assessed the bone volume fraction (BV/TV, %), trabecular thickness (TbTh, μm), trabecular number (TbN, mm^{-1}), trabecular connectivity density (Tb Conn Density, mm^{-3}) and structural model index (SMI). The SMI was measured to determine the prevalence of plate-like or rod-like trabecular structures, where 0 represents "plates" and 3 "rods" (5). For cortical bone at the femoral and tibial midshaft, we measured the cortical tissue volume (CtTV, mm^3), bone volume (CtBV, mm^3), the marrow volume (BMaV, mm^3) and the average cortical thickness (CtTh, μm).

RNA extraction and quantitative PCR. The whole femur was excised and both femur extremities were cut to remove the bone marrow from the diaphysis, by flushing with cold PBS. Femur diaphysis and extremities were immediately pulverized to a fine powder and homogenized in peqGold Trifast (peQLab Biotechnologie) using FastPrep System apparatus (QBiogene) to achieve quantitative RNA extraction. Total RNA was extracted and then purified on minicolumns (RNeasy Mini kit; Qiagen) in combination with a DNase treatment (RNase-free DNase Set, Qiagen) to avoid DNA contamination.

Single-stranded cDNA templates for quantitative real-time PCR (qRT-PCR) analyses were carried out using SuperScript III Reverse Transcriptase (Invitrogen) following the manufacturer's instructions. qRT-PCR were performed using predesigned TaqMan Gene Expression Assays (*GAPDH*: Mm00437762_m1; *Sost*: Mm00470479_m1; *Postn*: Mm00450111_m1; Applied Biosystems) consisting of two unlabeled primers and a FAM dye-labeled TaqMan MGB probe, and the correspondent buffer TaqMan Universal PCR Master Mix (Applied Biosystems). A Biomek 2000 robot (Beckman Coulter) was used for liquid handling (10 μL) in 384-well plates with three replicates per sample. The cDNA was PCR-amplified in a 7900HT SDS System and raw threshold-cycle (Ct) values were obtained from SDS 2.0 software (Applied Biosystems). Relative quantities (RQ) were calculated with the formula RQ = E − Ct using an efficiency (E) of 2 by default. For each gene the highest quantity was arbitrarily designated a value of 1.0. The mean quantity was calculated from triplicates for each sample and this quantity was normalized to the similarly measured mean quantity of the β2-microglobulin (GAPDH) normalization gene. Finally, normalized quantities

were averaged for three to four animals and represented as mean ± SEM.

Immunohistochemistry. The right and left tibiae were excised and subsequently fixed in 4% paraformaldehyde overnight at 4 °C. These tibiae were then decalcified in 19% EDTA and 4% phosphate-buffered formalin for 3 wk. The tibiae were then dehydrated in an ascending series of ethanol, cleared in Propar (Anatech), and embedded in paraffin blocks. Next, 10-μm-thick sections were cut from the blocks at the tibia midshaft level using a RM2155 microtome (Leica) and mounted on Superfrost Plus slides (Fisher Scientific). Sections were air-dried overnight at room temperature. Before staining, the sections were incubated at 60 °C for 1 h, deparaffinized in xylene, and rehydrated in a descending series of ethanol. Deparaffinized slides were pretreated in 3% hydrogen peroxide in methanol to quench endogeneous peroxidase and rinsed in tap water, followed by nonspecific avidin/biotin blocking (Vector Laboratories) according to the manufacturer's directions. All incubations took place in a humidified chamber. Additional protein blocking was accomplished with Protein Block-Serum Free (Dako). Using the Vectastain Elite ABC (Goat IgG) Kit (Vector Laboratories), the slides were incubated in 1.5% normal goat serum for 30 min at room temperature. The primary antibody (Goat anti-Periostin) (AF2955; R&D Systems) was diluted in Antibody Diluent (Dako) to a final concentration of 1:6,000 and incubated at 4 °C overnight. The following day, slides were rinsed in Wash Buffer (Dako) for 15 min on a rocker at room temperature and incubated in biotinylated goat anti-rabbit (Vectastain Kit) secondary antibody diluted 1:1,000 for 30 min at room temperature, followed by another rinse in Wash Buffer for 15 min on a rocker at room temperature. The ABC reagent from the Vector Kit was prepared according to the manufacturer's directions at a dilution of 1:250 and the slides were incubated in it for 30 min at room temperature and rinsed, as above, in Wash Buffer. All incubation steps were performed at room temperature and all rinse steps used the Dako Wash Buffer at room temperature on a rocker. The following protocol was used: incubation in streptavidin-HRP diluted at 1:100 for 30 min, and washed for 15 min. Slides were developed in a working solution of 3,3′-diaminobenzidine (DAB Substrate Kit for Peroxidase Kit; Vector Laboratories) prepared according to the manufacturer's directions for 10 min at room temperature. Following a final rinse in deionized water, the slides were counterstained in Weak Methyl Green and mounted in Cytoseal 60 (Richard-Allan Scientific). The same procedure was used to stain sclerostin by using primary antibody (Goat anti-sclerostin) (AF1589; R&D Systems). Positive Postn and Sost staining was quantified using a microscope interfaced with an image analysis system (Leica). Immunohistofluorescence of Sost and Postn was determined using anti-Sost conjugated to Fluor-488 (bright green fluorescence) and anti- Postn conjugated to Texas-red (red fluorescence) (Invitrogen). For the negative control, primary antibody incubation have been replace by Tris 0.1 M. Digital images were obtained using an upright microscope with a camera AxioCam MRc5 controlled by Axiovision AC software (Carl Zeiss MicroImaging).

Histomorphometry. To measure dynamic indices of bone formation, mice received subcutaneus injections of calcein (10 mg/kg; Sigma) 9 and 2 d before being killed. Femur were embedded in methylmethacrylate (Merck), and 20-μm-thick transversal sections of the midshaft were cut with a saw (FinOcut; Metkon Instruments), then sanded to 10-μm thickness and mounted unstained for evaluation of fluorescence. Five-micrometer-thick sagital sections were cut with a Leica Polycut E microtome (Leica) and stained with modified Goldner's trichrome; histomorphometric measurements were performed on the secondary spongiosa of the proximal tibia metaphysis and on the endocortical and periosteal bone surfaces in the middle of the tibia, using a Leica Q image analyzer at 40× magnification. All parameters were calculated and expressed according to standard formulas and nomenclatures (6): mineral apposition rate (MAR, μm/d), single labeled surface (sLS/BS, %), and double-labeled surface (dLS/BS, %). Mineralizing surface per bone surface (MS/BS, %) was calculated by adding dLS/BS and one-half sLS/BS. Bone formation rate (BFR/BS, $\mu m^3 \cdot \mu m^2 \cdot d$) was calculated as the product of MS/BS and MAR.

Testing of mechanical resistance. The night before mechanical testing, bones were thawed slowly at 7 °C and then maintained at room temperature. The length of the femur (distance from intermalleolar to intercondylar region) was measured using calipers with an integrated electronic digital display and the midpoint of the shaft was determined. The femur then was placed on the material testing machine on two supports separated by a distance of 9.9 mm and load was applied to the midpoint of the shaft, thus creating a three-point bending test. Between each preparation step, the specimens were kept immersed in physiological solution. The mechanical resistance to failure was tested using a servo-controlled electromechanical system (Instron 1114; Instron) with actuator displaced at 2 mm/min. Both displacement and load were recorded. Ultimate force [maximal load, measured in Newtons (N)], stiffness (slope of the linear part of the curve, representing the elastic deformation, N/mm), and energy (surface under the curve, N·mm) were calculated. Ultimate stress (N/mm^2) and Young's modulus (MPa) were determined by the equations previously described by Turner and Burr (7). Reproducibility was 5.8% for proximal femur, 3.3% for midshaft femur, and the coefficient of variation of paired sample measurements (left/right) was evaluated. Moment of inertia, indirect measurement of the bone resistance, was calculated by taking into account the medio-lateral and antero-posterior inner and outer diameter of the bone (8).

In Vitro PTH Experiments. ***UMR-106 culture.*** Osteoblast-like UMR-106 cells are the suitable model to study the relationship between Postn and Sost because they express these two proteins at high levels versus the low level of Sost expression in primary osteoblasts (9). Stock cultures of osteoblast-like UMR-106 cells were maintained and passage every 3–4 d when reaching confluence in DMEM/F12 media containing 10% FBS (10). Cells were plated in six-well plates at a concentration of 50,000 cells per well containing 2 mL of DMEM/F12 with 10% FBS. The cells were incubated during 7 d, medium was replaced every 2 d, and cells were exposed to PTH (bovine; Sigma-Aldrich) for 1, 2, 4, 6, 18, and 24 h at a dose of 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M, depending on the experiment, Postn-neutralizing antibodies (2 μg/mL) for 24 h (AF2955; R&D Systems), mouse recombinant periostin (4 μg/mL) (2955-F2; R&D Systems), and αVβ3 integrin antibody (2 μg/mL or 3 μg/mL) reactive with the vitronectin receptor αVβ3 complex, an RGD-directed adhesion receptor (MAB 1976; R&D Systems).

RNA was extracted for Postn, Sost, and MEF2C gene expression-level analysis relative to GAPDH. RNA extraction and conditions for quantitative PCR is described above. qRT-PCR were performed using predesigned TaqMan Gene Expression Assays (*MEF2C*: Mm01340842_m1*; Applied Biosystems).

To determine Postn and Sost changes in response to PTH at the protein level, Western blot analysis was performed. Cells were prepare and treated as described for the RNA; 24 h after PTH (10^{-7} M) treatment, UMR-106 cells were rinsed with ice-cold PBS and proteins were extracted in ice-cold RIPA buffer (50 mM Tris-HCl, pH 7.5;135 mM NaCl; 1% Triton X-100; 0.1% sodium deoxycholate; 2 mM EDTA; 50 mM NaF; 2 mM sodium orthovanadate; 10 μg/mL aprotinin; 10 μg/mL leupeptin, and 1 mM PMSF). Homogenates were incubated overnight at 4 °C, centrifuged, and supernatants were stored at −80 °C. Proteins were mixed with 5× laemilli sample buffer, boiled for 5 min, and subjected to 10% SDS-PAGE. Proteins were electroblotted to a nitrocellulose membrane, the membrane was blocked in 5% nonfat dry milk in Tris-buffered saline (TBS) with 0.1% tween-20 (TTBS) or for 2 h, and incubated in 0.1 μg/mL Postn primary antibody or Sost primary antibody overnight at 4 °C (11). The membrane was

washed with TTBS, incubated with 0.2 μg/mL of HRP-conjugated anti-goat IgG secondary antibody for 1 h at room temperature, and the chemiluminescent signal was detected using the ECL kit.

To assess immunocytochemistry, 24 h after PTH (10^{-7} M) treatment, UMR-106 cells were fixed, treated with 1% BSA, incubated with a goat anti-Postn, or a goat anti-Sost at a final concentration of 1:6,000, and incubated at 37 °C for 4 h. We than performed the same step as described above for the immunohistchemistry by using a Vectastain Elite ABC Kit and DAB Substrate Kit for Peroxidase staining.

Primary osteoblast culture and PTH. To assess the effects of PTH on osteoblast proliferation and differentiation, primary osteoblast cultures isolated from *Postn*$^{+/+}$ and *Postn*$^{-/-}$ newborn calvaria were exposed to PTH (10^{-7} M) in a medium permissive to mineralization. For this purpose, cells were harvested by sequential collagenase type II (3 mg/mL; Sigma-Aldrich) digestions of calvaria from 2- to 3-d-old wild-type or *Postn*$^{-/-}$ mice, half issued from male and half from female pups. Cells from the third to fifth digestions were pooled (12) and cultured in αMEM (Gibco), supplemented with 10% FCS (Amimed), antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL; Gibco), glutamine (200 mM; Gibco), amino acids (Amimed), and amphotericin B (0.25 μg/mL; Amimed). The cells were incubated at 37 °C with 5% CO_2 and media was changed every 2 d until they were 80% confluent.

To assess proliferation, primary osteoblasts were plated in 24-well plates at a concentration of 10,000 cells per well in EMEM media containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. Two, 4, and 6 d later, proliferation was assessed by thymidine measurement. Thymidine 3H was added in the medium 6 h before the cells were frozen at −80 °C and than counted on a scintillator.

To assess differentiation, differentiation factors were added (50 μg/mL ascorbic acid and 100 nM β-glycerolphosphate). Differentiation factors were added each time the media was changed. Alkaline phosphatase (ALP) staining was assessed on day 14, and mineral depositions (Alizarin red staining) were assessed on day 21. Experiments were repeated four times in triplicate. RNA was extracted from primary culture at days 14 and 28, respectively, for osteoblastic differentiation and osteocytic markers. Total RNA from *Postn*$^{+/+}$ and *Postn*$^{-/-}$ primary osteoblastic cells was extracted using TriPure (Roche), combined with DNase treatment (RNase-free DNase Set; Qiagen) and purification on RNeasy Minicolumn (Qiagen). Total RNA (2 μg) was reverse-transcribed using the high-capacity cDNA Archive Kit (Applied Biosystems) and diluted twofold. qRT-PCR (ABI PRISM 7000) was done as follows: 2 min at 50 °C, 10 min at 95 °C, and cycles of PCR consisting of 0.15 min at 95 °C and 1 min at 60 °C for 40 cycles. Reactions were performed in 25 μL containing 5 μL of cDNA, 12.5 μL of 2× TaqMan Universal PCR Master Mix, 1.25 μL of 20× mix of predesigned primers, and TaqMan MGB probes (FAM dye labeled, Assays-on-Demand products; Applied Biosystems) and H_2O up to 25 μL. qRT-PCR were performed using predesigned TaqMan Gene Expression Assays (*GAPDH*: Mm00437762_m1; *Alp1*: Mm00475834_m1; *RUNX2*: Mm00501878_m1; *OPN*: Mm00436767_m1; *Bglap*: Mm03413826_m1; *ATF-4*: Mm00515324_m1; *MEF2C*: Mm01340842_m1; *Phex*: Mm00448119_m1; *MEPE*: Mm02525159_m1, *Sost*: Mm00470479_m1; Applied Biosystems). mRNA level of each gene was normalized by GAPDH as an internal standard. Experiments were repeated three times in triplicate.

To assess migration, a scratch-wound (4-mm wide) was made across the diameter of the well using the end of a 200-μL pipette tip and scraped cells were removed by washing three times with PBS. The cells were maintained in EMEM media containing 2% FBS for the observation of the migration. For each well, images were taken at 0, 24, and 48 h after the wound, and the number of cells migrated into the wound space were manually counted in five fields per well.

To assess apoptosis by immunocytochemistry, 24 h after PTH (10^{-7} M) treatment, UMR-106 cells were fixed, treated with 1% BSA, incubated with a goat anticaspase 3 (R&D Systems) at a final concentration 1:6,000, and incubated at 37 °C for 4 h. We than performed the same step than described above for the immunohistochemistry by using a Vectastain Elite ABC Kit and DAB Substrate Kit for Peroxidase staining.

In the PTH experiment, bovine PTH (PTH 1–34) (10^{-7} M; Sigma) was incubates for 6 h before the cells were frozen for proliferation measurement. For ALP and Von Kossa staining, PTH was added in the medium. These different treatment cycles of 48 h were repeated in fresh medium supplemented by β-glycerophosphate. Experiments were repeated in triplicate.

To confirm that integrin signaling is functional in *Postn*$^{-/-}$ osteoblasts, we tested the effect of RGD peptides (H-Agr-Gly-Asp-Ser-OH, H-11550025), control RGD (H-Agr-Gly-Glu-Ser-OH, H-77450005), and recombinant periostin (2 μg/mL) on classic signaling P-Src, P-Fak, and P-PKD with a treatment time of 15 min. Western blot analysis was performed as described above.

Data analysis. We first tested the effects of PTH within groups (*Postn*$^{-/-}$ and *Postn*$^{+/+}$) by unpaired *t* tests. We then tested the effects of repeated measures within groups (saline/PTH) by a one-way ANOVA repeat measurements with the genotype used as a factor. To compare the effect of genotype and the response to treatments PTH, we used a two-way ANOVA. As appropriate, post hoc testing was performed using Fisher's protected least-squares difference (PLSD). The *P* of interaction between the genotype and treatments (mechanical stimulation or exercise) is only indicated when it was found to be significant. Differences were considered significant at $P < 0.05$. Data are presented as mean ± SEM.

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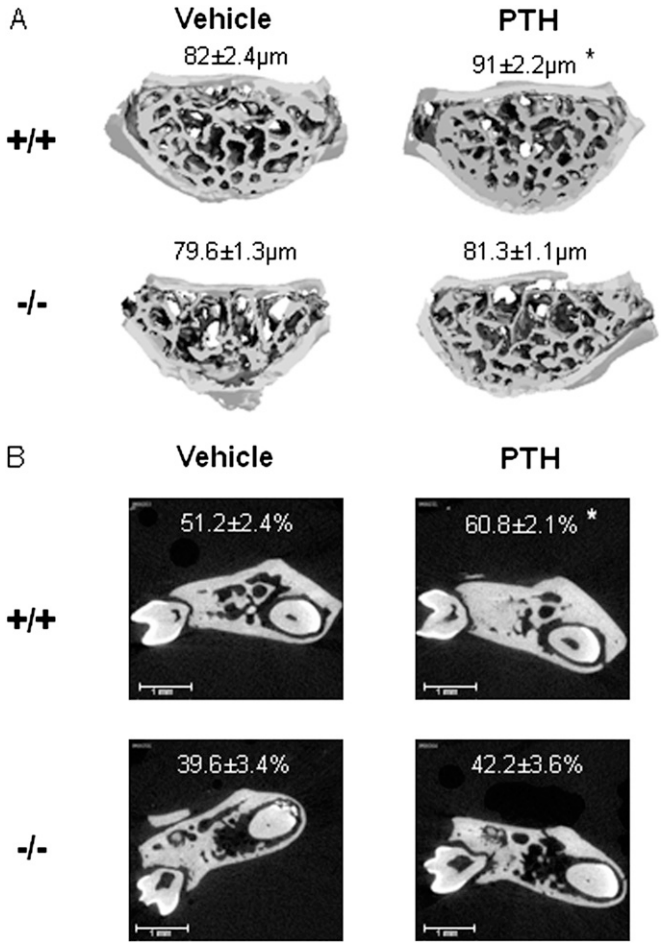

Fig. S1. Illustration of PTH effects on cortical and trabecular compartment of the vertebrae and on the alveolar bone of the jaw. (*A*) A 3D reconstruction of vertebral body. PTH increased bone volume fraction of the trabecular compartment independently of the genotype, whereas PTH increased cortical thickness only in the *Postn*$^{+/+}$ mice (cortical thickness mean ± SEM are given on the graph). (*B*) A 2D cross-sectional reconstruction of the right jaw. PTH increased alveolar volume fraction exclusively in the *Postn*$^{+/+}$ mice and not in the *Postn*$^{-/-}$ mice. Values of the alveolar bone volume mean ± SEM are given on the graph. *$P < 0.05$ significant difference vs. vehicle.

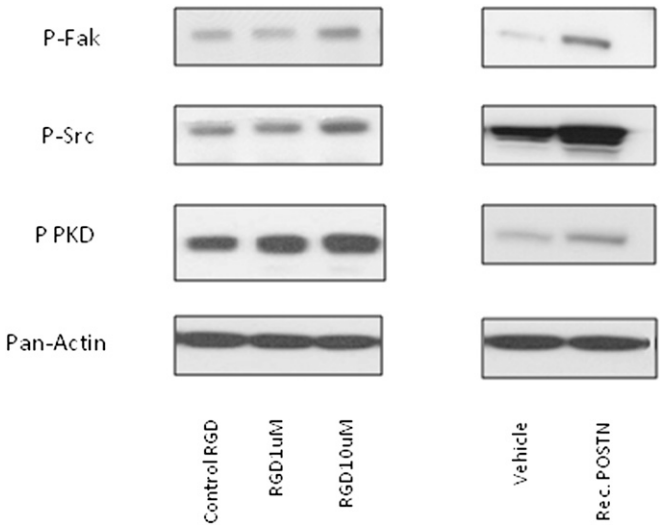

Fig. S2. Western blot analysis of P-Src, P-Fak, and P-PKD in response to RGD peptide (1 μM and 10 μM) and recombinant periostin (2 μg/mL) in *Postn*$^{-/-}$ osteoblast culture.

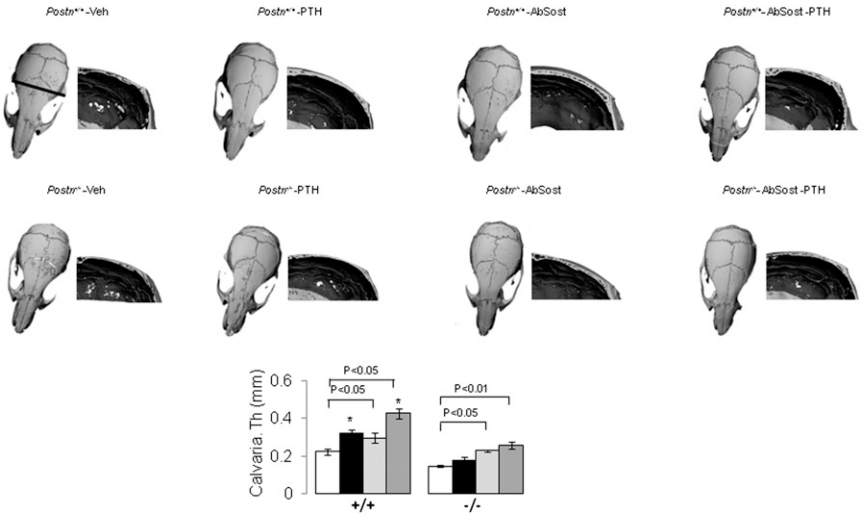

Fig. S3. Representative images of PTH effect combined or not to Sost antibody in *Postn*$^{+/+}$ and *Postn*$^{-/-}$ mice. Thre 3D reconstruction of the calvaria was obtained by microCT with tansverse sections taken 2-mm anterior to the junction between the fronto-parietal and sagittal sutures (indicated by the black line). *$P < 0.05$ significant effect of PTH vs. vehicle. Bars shows mean (± SEM). Open bars, Control-Ab+vehicle; closed bars, Control-Ab+PTH (40 μg·kg·d); light shadded bars, Sost-Ab+vehicle; dark shadded bars, Sost-Ab+PTH (40 μg·kg·d).

Table S1. Intermittent PTH effects on bone microarchitecture in *Postn*$^{+/+}$ and *Postn*$^{-/-}$ mice

	Postn$^{+/+}$		*Postn*$^{-/-}$			
	Vehicle	PTH	Vehicle	PTH	*P* treatment	*P* genotype
Vertibral body						
BV/TV (%)	19.4 ± 1.8	22.1 ± 1.9	15.9 ± 3.7	19.8 ± 1.1*	0.05	0.05
Conn D (1/mm^3)	132.8 ± 15.7	259.2 ± 26.8*	115.1 ± 7.5	269.5 ± 19.9*	0.001	0.85
TbN (1/mm)	3.9 ± 0.2	4.2 ± 0.2	3.7 ± 0.1	4.1 ± 0.2	0.12	0.79
TbTh (μm)	49.9 ± 1.6	52.4 ± 1.1*	47.1 ± 0.6	49.2 ± 0.4*	0.02	0.08
TbSp (mm)	0.26 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.75	0.75
SMI	0.86 ± 0.10	0.89 ± 0.16	1.12 ± 0.04	1.02 ± 0.12	0.35	0.85
CtTh (μm)	82 ± 2.4	91 ± 2.2*	79.6 ± 1.3	81.3 ± 1.1	0.01	0.01
Distal femur						
BV/TV (%)	7.1 ± 1.1	12.3 ± 1.5*	5.8 ± 0.5	10.5 ± 0.8**	0.22	0.0004
Conn D (1/mm^3)	52.3 ± 10.4	79.9 ± 15.1	36.9 ± 6.1	93.7 ± 11.1*	0.94	0.001
TbN (1/mm)	3.7 ± 0.2	4.3 ± 0.2*	3.42 ± 0.19	3.86 ± 0.22	0.16	0.03
TbTh (μm)	46.5 ± 2.1	54.7 ± 3.5*	43.9 ± 0.9	50.0 ± 0.2*	0.18	0.01
TbSp (mm)	0.27 ± 0.01	0.26 ± 0.02	0.30 ± 0.02	0.26 ± 0.02	0.65	0.31
SMI	3.2 ± 0.2	3.0 ± 0.1	3.1 ± 0.1	2.6 ± 0.1*	0.10	0.01
C.PoV/TV (%)	0.10 ± 0.01	0.09 ± 0.01	0.14 ± 0.02	0.27 ± 0.05*	0.10	0.01
C.PoTh (mm)	28 ± 2.2	36 ± 2.3	38 ± 3.2	62 ± 8.6*	0.05	0.01
Midshaft femur						
CtTV (mm^3)	1.12 ± 0.03	1.19 ± 0.03	1.03 ± 0.03	1.05 ± 0.03	0.003	0.06
CtBV (mm^3)	0.52 ± 0.02	0.59 ± 0.01**	0.50 ± 0.01	0.51 ± 0.01	0.005	0.02
CtTh (μm)	206 ± 5	228 ± 2***	201 ± 1	207 ± 2	0.0001	0.0001
BMaV (μm^3)	598 ± 17	599 ± 21	619 ± 60	619 ± 79	0.68	0.99

Bone microarchitecture analysis performed at vertebral body after 5 wk of PTH or saline treatment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ PTH effect vs. vehicle (unpaired *t* test), and *P* treatment and *P* genotype <0.05 vs. *Postn*$^{+/+}$ mice (Fishers PLSD, 2F-ANOVA) ($n = 8$ mice per group). Means ± SEM. BMaV, bone marrow volume; BV/TV, bone volume on tissue volume; Connec D, connectivity density; C.PoV/TV, cortical porosity; C.PoTh, cortical pore thickness; CtTh, cortical thickness; SMI, structural model index; TbN, trabecular number; TbSp, trabecular separation; TbTh, trabecular thickness.

Table S2. Intermittent PTH effects on remodelling indices at cortical and trabecular bone surfaces in *Postn*$^{+/+}$ and *Postn*$^{-/-}$ mice

	Postn$^{+/+}$		*Postn*$^{-/-}$			
	Vehicle	PTH	Vehicle	PTH	*P* treatment	*P* genotype
Periosteal						
Ps MAR (μm/d)	0.495 ± 0.13	0.867 ± 0.11**	0.204 ± 0.13	0.217 ± 0.10	0.12	0.001
Ps BFR/BPm ($\mu m^2 \cdot \mu m \cdot d$)	0.001 ± 0.0003	0.006 ± 0.001**	0.0008 ± 0.0008	0.001 ± 0.004	0.001	0.002
Ps MPm/BPm (%)	0.169 ± 0.04	0.663 ± 0.08**	0.156 ± 0.09	0.268 ± 0.05	0.006	0.002
Endocortical						
Ec MAR (μm/d)	0.228 ± 0.115	0.986 ± 0.095**	0.181 ± 0.116	0.570 ± 0.294*	0.16	0.001
Ec BFR/BPm ($\mu m^2 \cdot \mu m \cdot d$)	0.001 ± 0.001	0.007 ± 0.001**	0.001 ± 0.001	0.003 ± 0.001	0.01	0.005
Ec MPm/BPm (%)	0.315 ± 0.081	0.728 ± 0.142**	0.195 ± 0.082	0.365 ± 0.05	0.02	0.009
Trabecular						
Tb MAR (μm/d)	1.04 ± 0.10	1.61 ± 0.18**	0.78 ± 0.12	1.06 ± 0.07*	0.004	0.005
Tb BFR/BS ($\mu m^2 \cdot \mu m^3 \cdot d$)	0.44 ± 0.03	0.67 ± 0.13	0.31 ± 0.09	0.66 ± 0.16	0.01	0.51
Tb MS/BS (%)	44.16 ± 2.57	43.60 ± 6.15	40.08 ± 6.27	47.86 ± 3.47	0.44	0.98
sLS/BS (%)	2.37 ± 0.22	2.78 ± 0.40	2.59 ± 0.28	2.67 ± 0.49	0.65	0.32
dLS/BS (%)	3.36 ± 0.40	3.91 ± 0.60	2.94 ± 0.45	3.45 ± 0.48	0.42	0.55
OcS/BS (%)	4.44 ± 1.22	7.88 ± 2.01	5.30 ± 1.11	11.30 ± 0.92**	0.004	0.16
OcN/T.Ar (mm)	18.3 ± 5.9	33.3 ± 8.4	28.8 ± 7.3	40.5 ± 5.9	0.07	0.22

Histomorphometric analysis performed at the femur after 5 wk of PTH or saline treatment. *$P < 0.05$, **$P < 0.01$ PTH effect vs. vehicle (unpaired *t* test), and *P* treatment and *P* genotype <0.05 vs. *Postn*$^{+/+}$ mice (Fishers PLSD, 2F-ANOVA) ($n = 8$ mice per group). Means ± SEM. BFR, bone formation rate; BPm, bone perimeter; BS, bone surface; dLS, double-labeled surface; Ec, endocortical; MAR, mineral apposition rate; MPm, mineralization perimeter; MS, mineralization surface; OcN, osteoclast number; OcS, osteoclast surface; Ps, periosteum; sLS, single-labeled surface; Tb, trabecular; T.Ar, tissue area.

Table S3. Characterization of femoral biomechanical properties in response to intermittent PTH in *Postn*$^{+/+}$ and *Postn*$^{-/-}$ mice

	Postn$^{+/+}$		*Postn*$^{-/-}$			
	Vehicle	PTH	Vehicle	PTH	*P* treatment	*P* genotype
Ultimate force (N)	19.10 ± 0.69	22.10 ± 0.93*	19.22 ± 1.10	19.80 ± 0.83	0.05	0.25
Stiffness (N/mm)	59.77 ± 3.14	70.21 ± 2.64*	62.48 ± 5.45	66.61 ± 3.06	0.05	0.905
Displacement (mm)	0.92 ± 0.03	1.21 ± 0.06***	1.03 ± 0.09	1.06 ± 0.05	0.01	0.85
Total Energy ($N - mm$)	5.10 ± 0.27	4.52 ± 0.23	4.50 ± 0.39	4.50 ± 0.13	0.33	0.29
Moment of inertia (mm^4)	0.118 ± 0.006	0.138 ± 0.005*	0.099 ± 0.06	0.107 ± 0.004	0.01	0.001

Biomechanical analysis performed at the midshaft femur after 5 wk of PTH or saline treatment. *$P < 0.05$, ***$P < 0.001$ PTH effect vs. vehicle (unpaired *t* test), and *P* treatment and *P* genotype <0.05 vs. *Postn*$^{+/+}$ mice (Fishers PLSD, 2F-ANOVA) ($n = 8$ mice/group). Means ± SEM.