Table S1. Glossary

Reagent	Company	Function
NaHS	Sigma-Aldrich Corporation	H_2S donor; At temperatures above 360 K, NaHS adopts the NaCl structure. Below 360 K, NaHS shows a rhombohedral structure forms.
hydroxylamine (HA)	Sigma-Aldrich Corporation	Inhibitor for CBS
DL-Propargylglycine (PAG)	Sigma-Aldrich Corporation	Inhibitor for CSE
GYY4317	Sigma-Aldrich Corporation	H_2S donor (slow-releasing)
Zn acetate	Sigma-Aldrich Corporation	Reagent for analyzing H_2S concentration
N, N-dimethyl-p-phenylenediamine sulfate	Sigma-Aldrich Corporation	Reagent for analyzing H_2S concentration
DL-Dithiothreitol (DTT)	Sigma-Aldrich Corporation	Increasing the number of free sulfhydryl
Diamide (DM)	Sigma-Aldrich Corporation	Decreasing the number of free sulfhydryl
MTSES	Affymetrix	A nonpermanent free sulfhydryl inhibitor, reduced sulfhydryl number outside the cell membrane
CHIR-99021	Sigma-Aldrich Corporation	GSK3β inhibitor
YS-49 monohydrate	Sigma-Aldrich Corporation	PI3K activator
LY-294,002 hydrochloride	Sigma-Aldrich Corporation	Phosphoinositide 3-kinase (PI3K) inhibitor
prostratin	Sigma-Aldrich Corporation	PKC activator
SKF-96365	Sigma-Aldrich Corporation	Calcium channel inhibitor
Capsazepine	Abcam Biochemicals	Calcium channel inhibitor
2-aminoethyl diphenylborinate (2APB)	Sigma-Aldrich Corporation	Calcium channel inhibitor
Ellman's reagent (DTNB)	Thermal Scientific Inc.	Selectively interacting with free thiols
L-cysteine HCl monohydrate	Thermal Scientific Inc.	Prepare standard sample for Ellman's reagent
M50 Super 8×TOPflash,	Addgene	Luciferase activity to detect the canonical Wnt/ β-catenin pathway
M51 Super 8×FOPflash	Addgene	TOPflash mutant
Gö 6976	EMD Millipore	Protein kinase C (PKC) inhibitor
Capsaicin	EMD Millipore	Calcium channel activator
PD325901	EMD Millipore	Extracellular signal regulated kinase (Erk) inhibitor
maleimide	Invitrogen Corporation	Selectively interacting with sulfhydryl

		groups of cysteines, labeling both sulfhydrated and unsulfhydrated cysteines, and no reacting with nitrosylated or oxidized cysteines.
Fura 2 AM	Invitrogen Corporation	Analyzing calcium flux

Supplementary Table1. Glossary used for Figures 1, 3, 4, 5,6, 7.

Table S2. *In Vivo* Used Chemical Agents

Chemical	Function	Dose	Total times	Experiment
NaHS	H_2S donor	200 μg/mouse (10 mg/kg)	1	Analyze H_2S level in mouse serum
GYY4317	H_2S slow releasing donor	1 mg/mouse (50 mg/kg)	14	$CBS^{+/-}$ mice rescue
HA	CBS inhibitor	100 μg/mouse (5mg/kg)	14	Analyze H_2S level in mouse serum; mimic CBS deficiency in C57BL6 mice
PAG	CSE inhibitor	100 μg/mouse (5mg/kg)	14	Analyze H_2S level in mouse serum; mimic CSE deficiency in C57BL6 mice

Supplementary Table 2. Chemical agents used for Figures 4, 5.

Supplementary Experimental Procedures

Genotyping. Primers used for genotyping include oIMR0263: GCCTCTGTCTGCTAACCTA; oIMR0264: GAGGTCGACGGTATCGATA; oIMR0655:GAAGGACAACCAACCACTGG; and oIMR3646:ATGGCCTGAGATTGCAAGG.

Antibodies. Anti-active β-catenin and β-catenin antibodies were purchased from Millipore (Temecula, CA). Anti-CD73-PE antibody was purchased from BD Bioscience (San Jose, CA). Unconjugated anti-Runx2 was purchased from Calbiochem Inc. (La Jolla, CA). Anti-Erk1/2, anti-phospho-Erk1/2, anti-PKCα, anti-phospho-PKCalpha/beta II, anti-GSK3β, anti-phospho - GSK3β, anti-Akt, and anti-phospho Akt antibodies were purchased from Cell Signaling Inc. (San Francisco, CA). Anti-β-actin antibody was purchased from Sigma-Aldrich Corporation (St. Louis, MO). Alexa Fluor® 488 C5 maleimide was purchased from Invitrogen Corporation (Carlsbad, CA). Fura 2 AM was purchased from Invitrogen Corporation (Carlsbad, CA). Unconjugated antibody to ALP and protein A/G PLUS-agarose were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Unconjugated antibodies to cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and TRPV6 were purchased from Abcam Inc. (Cambridge, MA).

Chemicals. Inhibitor for CBS (hydroxylamine, HA) and CSE (DL-Propargylglycine, PAG), H_2S donor NaHS, 2-aminoethyl diphenylborinate (2APB), SKF-96365, PKC activator (prostratin), DL-Dithiothreitol (DTT), Diamide (DM), Zn acetate, N, N-dimethyl-p-phenylenediamine sulfate, GSK3β inhibitor (CHIR-99021), PI3K activator (YS-49 monohydrate) and phosphoinositide 3-kinase (PI3K) inhibitor (LY-294, 002 hydrochloride) were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Protein kinase C (PKC) inhibitor (Gö 6976), capsaicin and extracellular signal regulated kinase (Erk) inhibitor (PD325901) were purchased from EMD Millipore (Billerica, MA). MTSES were purchased from Affymetrix (Santa Clara, CA). Capsazepine was purchased from Abcam Biochemicals (Cambridge, MA). Ellman's reagent (DTNB) and L-cysteine HCl monohydrate were purchased from Thermal Scientific Inc. **(**Odessa, Texas). M50 Super 8×TOPflash and M51 Super 8×FOPflash (TOPflash mutant) were purchased from Addgene (Cambridge, MA).

Kits. Lipofectamine™ RNAiMAX and BrdU staining kits were purchased from Invitrogen. ProteoExtract® Transmembrane Protein Extraction Kit (TM-PEK) was purchased from EMD Millipore (Billerica, MA).

Flow cytometric analysis of mesenchymal stem cell surface molecules. 0.1-0.2×10^6 BMMSCs were incubated with 1 μg of PE-conjugated antibodies or isotype-matched control IgGs (Southern Biotech, Birmingham, AL) at 4^{o}C for 45 min. Samples were analyzed by FACS^{Calibur} flow cytometer (BD Bioscience, San Jose, CA). For dual color analysis, the cells were treated with PE-conjugated and FITC-conjugated antibodies or isotype-matched control IgGs (each 1 μg). The cells were analyzed on FACS^{Calibur} (BD Bioscience).

Cell proliferation assay. Proliferation of each mesenchymal stem cell population was performed by bromodeoxyuridine (BrdU) incorporation assay. Briefly, BMMSCs (1.0×10^4 cells/well) were seeded on 2-well chamber slides (Nunc) and cultured for 2-3 days. The cultures were incubated with BrdU solution (1:100) (Invitrogen) for 20 hours and stained with a BrdU staining kit (Invitrogen) according to the manufacturer's instructions. BrdU-positive and total cell numbers were counted in ten image areas per subject. The number of BrdU-positive cells was indicated as a percentage of the total number of cells. The BrdU assay was repeated in 4 or 5 independent samples for each experimental group.

In vitro **adipogenic differentiation assay.** For adipogenic induction, 500 nM isobutylmethylxanthin (Sigma), 60 μM indomethacin (Sigma), 500 nM hydrocortisone (Sigma), 10 μg/ml insulin (Sigma) and 100 nM L-ascorbic acid phosphate were added into the culture medium. Ten days after the induction, the cultured cells were stained with Oil Red O, and positive cells were quantified using NIH ImageJ. Total RNA was also isolated from cultures after 10-day induction for further experiments. Expression of the adipogenic lineage-specific genes *LPL* and *PPARγ2* was assayed by RT-PCR.

In vitro **osteogenic differentiation assay.** BMMSCs were cultured under osteogenic culture conditions in media containing 2 mM β-glycerophosphate (Sigma), 100 μM L-ascorbic acid-2-phosphate and 10 nM dexamethasone (Sigma). Four weeks after osteogenic induction, the cultures were stained with alizarin red. Mineralized nodule areas were quantified using NIH

ImageJ. Total proteins were isolated from cultures after 14 days of induction for further experiments. Expression of Runx2 and ALP were assayed by Western blotting analysis.

BMMSC-mediated bone formation *in vivo*. Approximately 4.0×10^6 BMMSCs were mixed with hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic particles (40 mg, Zimmer Inc., Warsaw, IN) as a carrier and subcutaneously implanted into the dorsal surface of 8- to 10-week-old immunocompromised mice. At eight weeks post-implantation, the implants were harvested, fixed in 4% paraformaldehyde and then decalcified with 5% EDTA (pH 7.4), followed by paraffin embedding. The 6 μm paraffin sections were stained with hematoxylin and eosin (H&E) and analyzed by NIH ImageJ. Five fields were selected, and the newly formed mineralized tissue area in each field was calculated and shown as a percentage of the total tissue area.

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis. Total RNA was isolated from the cultures using SV total RNA isolation kit (Promega, Madison, WI) according to the manufacturer's instructions. The cDNA was synthesized from 100 ng of total RNA using Superscript III (Invitrogen). PCR was performed using gene-specific primers and Platinum PCR Supermix (Invitrogen). The specific primers used in RT-PCR were as follows: Peroxisome proliferator-activated receptor γ2 (*PPARγ2*, GenBank accession no. NM_011146): sense 5'-GCTGTTATGGGTGAAACTCTG-3' (nucleotides 40–60) and antisense 5'-ATAAGGTGGAGATGCAGGTTC-3' (nucleotides 370–390); lipoprotein lipase (*LPL*, GenBank accession no. NM_008509): sense 5'-GGGCTCTGCCTGAGTTGTAG-3' (nucleotides 1096–1115) and antisense 5'-AGAAATTTCGAAGGCCTGGT-3' (nucleotides 1274–1293); glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*, GenBank accession no. NM_008084): sense 5'-CACCATGGAGAAGGCCGGGG-3' (nucleotides 353–372) and antisense 5'-GACGGACACATTGGGGGTAG-3' (nucleotides 751–770).

Western blotting analysis. Total protein was extracted using M-PER mammalian protein extraction reagent (Thermo, Rockford, IL). Protein was applied and separated on 4-12% NuPAGE gel (Invitrogen) and transferred to Immobilon™-P membranes (Millipore). The membranes were blocked with 5% non-fat dry milk and 0.1% Tween 20 for 1 hour, followed by incubation with the primary antibodies (1:100-1000 dilution) at 4^oC overnight. Horseradish peroxidase-conjugated IgG (Santa Cruz Biosciences; 1:10,000) was used to treat the membranes for 1 hour and enhanced with a SuperSignal® West Pico Chemiluminescent Substrate (Thermo).

The bands were detected on BIOMAX MR films (Kodak, Rochester, NY). Each membrane was also stripped using a stripping buffer (Thermo) and reprobed with anti-β-actin antibody to quantify the amount of loaded protein.

siRNA. 0.5×10^6 BMMSCs were seeded on a 6-well culture plate. siRNAs were used to treat the BMMSCs according to the manufacturer's instructions. siRNA kits for TRPC1, TRPC3, TRPC7, TRPV1, TRPV4 and TRPM2, 4, 8 were purchased from Santa Cruz Biosciences (Santa Cruz, CA).

Bone analyses by micro-computed tomography (microCT) and peripheral quantitative CT (pQCT). Distal femoral metaphyses were analyzed by microCT (μCT-20; SCANCO USA, Inc.). Scanning regions were confined to secondary spongiosa and were ~0.30 mm in thickness. Using 2-dimensional images, a region of interest was manually drawn near the endocortical surface. Cancellous bone morphometric indices were assessed using 3-dimensional image reconstructions and included bone volume relative to tissue volume (BV/TV, %), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp). pQCT analysis of the distal femora was performed using a XCT Research M (Stratec; Norland Co.). Briefly, scans were obtained at 2.25 and 2.75 mm from the distal condyles and cancellous BMD. Machine cancellous BMD precision (based on manufacturer data) is ±3 mg/cm^3, while the coefficient of variation in our laboratory based on repeat scans was 2.26%.

Histological analysis. Femurs were fixed with 4% paraformaldehyde, decalcified with 10% EDTA (pH 8.0) and embedded in paraffin. Sections were deparaffinized and stained with hematoxylin and eosin (H&E). For quantification of trabecular bone, five representative images were analyzed by using NIH ImageJ. The results were shown as the percentage of trabecular bones per total bone area.

Measurement of Ca^{2+} image. 0.2×10^6 BMMSCs were seeded onto 35 mm culture dishes and cultured for 24 hours at 37 ^oC in 5% CO_2. Then, the cells were loaded with fura-2 AM (Invitrogen) and incubated for 1 h at 37 ^oC in 5% CO_2 in the dark. Ca^{2+} levels were measured from the ratio of emission in response to excitation at 340 and 380 nm on an Olympus Optical IX71 microscope.

Measurement of H_2S. Serum and culture supernatant were mixed with 0.25 ml Zn acetate (1%)

and 0.45 ml water for 10 minutes at room temperature. TCA (10%; 0.25 μl) was then added and centrifuged (14,000 **g**; 10 minutes; 4°C). The supernatant was then collected and mixed with N,N-dimethyl-p-phenylenediamine sulfate (20 μM) in 1.2 M HCl and $FeCl_3$ (30 μM) in 1.2 mol/L HCl. After 20 minutes, absorbance was measured at 650 nm.

TOPflash and FOPflash. M50 Super 8×TOPflash was used as a luciferase reporter of β-catenin-mediated transcriptional activation, which had the TCF/LEF binding sites upstream of the luciferase open reading frame. The appropriate control plasmid is clone M51 Super 8×FOPflash, which has mutant TCF/LEF binding sites. 0.1×10^6 BMMSCs were seeded onto a 24-well culture plate and cultured for 24 hours. 10 ng/well TOPflash or FOPflash and 4 ng/well renilla luciferase were transfected into BMMSCs using a Lipofectamine™ RNAiMAX kit (Invitrogen). After 15 h, the plates were assayed for firefly luciferase and renilla luciferase activities using the Dual-Luciferase® Reporter Assay System (Promega).

Ca^{2+} channel sulfhydration analysis by LC/MS/MS. 1×10^7 BMMSCs were incubated with 100 μM NaHS for 15 min at 37 ^{o}C. Membrane proteins were extracted using a ProteoExtract® Transmembrane Protein Extraction Kit (Millipore). To remove unreacted NaHS and eliminate artificial modifications after DTT treatment, samples were desalted using Zeba Spin Desalting Columns (Thermo Scientific). Desalted samples were denatured, reduced with DTT, alkylated with Iodacetamide, and digested with trypsin as described previously (Zhou et al., 2011). Negative control experiments were conducted identically, using all reagents except NaHS. Samples were analyzed using an LC/MS system consisting of an Eksigent NanoLC Ultra 2D (Dublin, CA) and Thermo Fisher Scientific LTQ Orbitrap XL (San Jose, CA). Briefly, peptides were separated in a 10 cm column (75 μm inner diameter) packed in-house with 5 μm C18 beads on an Eksigent NanoLC Ultra 2D system using a binary gradient of buffer A (0.1 % formic acid) and buffer B (0.1% formic acid and 80% ACN). The peptides were loaded directly with buffer A without any trapping column at a flow rate of 300 nL/min. Elution was carried out at a flow rate of 250 nL/min, with a linear gradient from 10% to 35% buffer B for 95 min followed by 50% B for 15 min. At the end of the gradient, the column was washed with 90% B and equilibrated with 5% B for 10 min. The eluted peptides were sprayed into the LTQ Orbitrap XL. The source was operated at 2.1-2.25 kV, with no sheath gas flow, with the ion transfer tube at 250 °C. MS spectra in the range of m/z 350–2000 were acquired in the orbitrap at a FWHM resolution of

60,000 after accumulation to an AGC target value of 500,000 in the linear ion trap with 1 microscan. For peptide sequencing and modification site localization, the same precursors selected for fragmentation by CID and fragment ions were analyzed in the liner ion trap. The five most abundant precursor ions were selected for fragmentation by CID. The instrument was operated in data-dependent acquisition mode, whereby five CID data-dependent MS/MS scans succeeded the high resolution MS scan. For all sequencing events, dynamic exclusion was enabled to minimize repeated sequencing. Peaks selected for fragmentation more than once within 60 s were excluded from selection (10 ppm window).

Proteome Discoverer 1.3 (Thermo Fisher Scientific) was used for protein identification using Sequest algorithms. The following criteria were followed. For MS/MS spectra, the variable modifications were carbamidomethylation modification of S-sulfhydration of cysteine, carbamidomethylation of cysteine and oxidation of methionine with a maximum of four modifications. Searches were conducted against Uniprot or an in-house customer database. Up to two missed cleavages were allowed for protease digestion, and peptides had to be fully tryptic. MS1 tolerance was 10 ppm, and MS2 tolerance was set at 0.8 Da. Peptides reported via search engine were accepted only if they met the false discovery rate of 1%. In the absence of a fixed cutoff score threshold, spectra were accepted until the 1% FDR rate was reached. Only peptides with a minimum length of six amino acids were considered for identification. We also validated the identifications by manual inspection of the mass spectra.

Sulfhydration assay using maleimide. This assay was designed based on principles described previously (Aracena-Parks et al., 2006; Sen et al., 2012). Membrane proteins were collected by ProteoExtract® Transmembrane Protein Extraction Kit (TM-PEK) (EMD Millipore, Billerica, MA). Supernatant-enriched membrane protein was transferred to a fresh tube on ice and incubated with Alexa Fluor® 488 C5 maleimide (green, 10 μM, Invitrogen, final concentration) for 2 hours at 4 °C with occasional gentle mixing. 1.0 μg of the appropriate control IgG, together with 20 μl of resuspended volume of protein A/G PLUS-agarose, was added to precleared lysate and incubated at 4 °C for 30 minutes. Pellet beads were centrifuged at 2,500 rpm for 5 minutes at 4 °C, and the supernatant was transferred to a fresh centrifuge tube on ice. Approximately 100 μg of total cellular protein were used in each group. Primary antibody (1:50) was added and incubated for 1 hour at 4° C. 20 μl of resuspended volume of protein A/G PLUS-agarose were

added at 4° C overnight. Immunoprecipitates were collected by centrifugation at 2,500 rpm for 5 minutes at 4° C. The beads were pelleted and washed with RIPA buffer and then treated with or without DTT (1 mM, final concentration) for 1 hr at 4°C. Beads were pelleted, washed and resuspended in 40 µl of electrophoresis sample buffer. The samples were boiled at 95 °C for 3 minutes, and 20 µl aliquots were applied and separated on 4-12% NuPAGE gel (Invitrogen), then transferred to Immobilon™-P membranes (Millipore), which were scanned with the Li-COR Odyssey system. The intensity of green fluorescence of the protein was quantified with software attached to the Odyssey system. These membranes were also employed for Western blotting with anti-protein antibody, followed by incubation with the secondary antibodies at 4^{o}C overnight. The bands were detected on BIOMAX MR films (Kodak, Rochester, NY) after enhancement with a SuperSignal® West Pico Chemiluminescent Substrate (Thermo).

Osteoclast culture and activity assay. Bone marrow cells (BMCs) were collected by injection of PBS throughout the entire marrow cavity of the tibiae and femora of 8-week-old mice, and 0.5×10^{6} BMCs were suspended in αMEM (Invitrogen) containing 15% heat-inactivated FBS (Equitech-Bio), L-glutamine (Invitrogen), penicillin and streptomycin (Invitrogen) and 20 ng ml^{-1} macrophage colony-stimulating factor 1 (M-CSF, R&D) in a 24-well plate for 48 hours. Adherent cells were harvested and cultured with 20 ng ml^{-1} M-CSF (R&D) and 50 ng ml^{-1} sRANKL (PeproTech) for another 4 days, followed by fixation and TRAP staining according to the manufacturers' protocols.

Generation of TRPV6 vector point mutant constructs. *Trpv6* mammalian expression construct (Thermo Scientific) was used as a template to generate point mutants at C172S and C329S by PCR using Pfu polymerase, followed by the DpnI cloning method. C172S point mutant construct was used as a template to generate second point C329S mutant for double site mutant construct. All clones were confirmed by DNA sequencing. Primers used to generate point mutant constructs were as followed, TRPV6 C172S F: CCTTTGCTGCCTCTGTGGGTAGTGAG; TRPV6 C172S R: CTCACTACCCACAGAGGCAGCAAAGG; TRPV6 C329S F: GGCCCTACTTCTCCGTGCTGGGTGC; TRPV6 C329S R: GCACCCAGCACGGAGAAGTAGGGCC.

Statistics. Student's *t*-test was used to analyze statistical difference. P values less than 0.05 were considered significant.

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