SI Experimental Procedures

Mice

All mice were kept under standardized conditions with water and food *ad libitum* in specialized pathogen-free animal facilities at the University of Ulm. Procedures for performing animal experiments were in accordance with and approved by the authorities for ethical permission (the Regierungspräsidium in Tübingen, Germany). *Men1*^{Runx2Cre}, *Men1*^{OsxCre}, *Men1*^{gtRosaCreERT2}, *Men1*^{LysMCre}, *Men1*^{Dmp1Cre}, *Men1*^{Dmp1Cre} × Rosa$^{mT/mG}$, and Runx2Cre × Rosa$^{mT/mG}$ mice were generated by intercrossing *Men1*^{flox} mice [13] with Tg(Runx2-icre)1Jtuc mice [14], Tg(Sp7-tTA,tetO-EGFP/cre)1Amc mice [17], C57BL/6-Gt(ROSA)26Sor$^{tm9(Cre/ESR1)Arte}$ mice (Taconic Artemis, Köln, Germany), Lyz2$^{tm1(cre)Ifo}$ mice [16], Tg(Dmp1-cre)1Jqfe mice [18], and Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J mice [15], respectively.

Histomorphometry

Static and dynamic histomorphometry was performed on undecalcified and decalcified lumbar vertebral and femoral sections of mice receiving dual calcein (Sigma-Aldrich, St. Louis, USA) i.p. injections, as described previously [25, 26]. Osteomeasure software was used for analysis (Osteometrics, Decatur, USA).

Micro computed tomography (micro CT)

Femurs, tibiae, and lumbar vertebrae were analyzed using a SkyScan 1174 compact micro CT or SkyScan 1176 *in vivo* micro CT (Bruker, Billerica, USA) equipped with an X-ray tube working at 50–80 kV/100 µA. Resolution was 6–9 µm, rotation step was set at 0.40° or 1°,

and a 0.5-mm aluminum filter was used. For reconstruction of femurs, the region of interest was defined 0.3 mm from the distal growth plate into the diaphysis spanning 1.8 mm. Trabecular bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th.), trabecular separation (Tb.Sp.), trabecular number (Tb.N.), cortical thickness (Cs.Th.), cortical closed porosity, and cortical bone perimeter (B.Pm.) were determined according to the guidelines issued by the ASBMR Histomorphometry Nomenclature Committee [24, 25].

Osteoblast differentiation from stromal and calvarial cells

Stromal cells were isolated from inguinal white adipose tissue of *Men1*^{gtRosaCreERT2} mice (6–8 weeks) by digestions for 30 min at 37 ℃ in isolation buffer containing 100 mM HEPES (Sigma-Aldrich), 120 mM NaCl (Merck, Darmstadt, Germany), 4.8 mM KCl (Sigma-Aldrich), 1 mM $CaCl_2$•$2H_2O$ (Sigma-Aldrich), 4.5 mM glucose (Merck), 1.5% albumin (Sigma-Aldrich), and 0.2% collagenase II (Roche, Rotkreuz, Switzerland). The stromal vascular fraction was collected after filtration and centrifugation and subsequently cultivated as described for calvarial cells below.

Bone marrow stromal cells were isolated from femur and tibia of *Men1*^{gtRosaCreERT2} mice (6–8 weeks). Bone marrows were flushed out and cells were seeded at a density of 1×10^6 cells per cm^2. Seventy-two hours later, cells were washed three times with medium (DMEM containing 20% fetal bovine serum and 1% penicillin/streptomycin) and subsequently cultivated as described for calvarial cells below.

Primary osteoblasts isolated from calvaria of neonatal *Men1*^{gtRosaCreERT2} mice (postnatal day 3–5) by sequential digestions were cultivated as previously described [14]. The cells were exposed to 1 μM 4-OHT (Sigma-Aldrich) for 3 days and subsequently subjected to osteogenic induction medium (alpha-MEM containing 10% fetal bovine serum and 1%

penicillin/streptomycin, supplemented with 100 mg/ml sodium ascorbate and 5 mM beta-glycerophosphate; Sigma-Aldrich) and/or 100 ng/ml hBMP2 (R&D Systems, Minneapolis, USA). Osteoblast differentiation was determined by alkaline phosphatase and alizarin red (both from Sigma-Aldrich) staining at indicated time points.

Osteoclast differentiation

BMCs were isolated from femurs of 2- to 3-month-old mice and 200,000 cells (diameter > 5 µm) were seeded per well in 96-well plates. Differentiation of osteoclasts was performed in alpha-MEM containing 10% fetal bovine serum and 1% penicillin/streptomycin, supplemented with 50 ng/ml RANKL (PeproTech, Rocky Hill, USA) and 20 ng/ml M-CSF (R&D Systems) for 5 or 7 days, with the medium changed every 2 or 3 days. TRAP staining was performed using a TRAP-kit (Sigma-Aldrich) and TRAP-positive cells with more than three nuclei were counted as osteoclasts. Osteoclast area was quantified using Osteomeasure software.

For conditioned medium-transfer experiments, conditioned medium from *Men1*-deficient primary osteocyte-enriched fractions or control primary osteocyte-enriched fractions was diluted 1:1 in culture medium.

Osteocyte isolation

Primary osteocyte-enriched fractions from *Men1*^{gtRosaCreERT2} mice were isolated as previously described [27]. Osteocytes were extracted by sequential digestions with collagenase and EDTA (AppliChem, Darmstadt, Germany). Fractions 3–5 of the sequential digest were considered as osteoblasts. Fractions 7–9 and cells derived from bone particles were pooled and considered as osteocytes. After one passage, 8,000 cells per well were seeded into

collagen-coated 96-well plates (Greiner Bio-One, Kremsmünster, Austria) and treated with 1 µM 4-OHT for 3 days.

Co-culture experiments

BMCs were isolated from wild-type mice and 200,000 cells per well were added on top of primary osteocyte-enriched fractions or primary osteoblasts cultivated in co-culture medium (alpha-MEM supplemented with 10 nM 1,25-dihydroxyvitamin D3 (Sigma-Aldrich) and 20 ng/ml M-CSF). For pit assay, bone slices (Immunodiagnostic Systems, Tyne & Wear, UK) were used. Cells were removed from bone slices with 1 M ammonium hydroxide overnight and the resorption pits were visualized using 0.5% (w/v in H_2O) toluidine blue.

For conditioned medium-transfer experiments, conditioned medium from *Men1*-deficient primary osteocyte-enriched fractions or control primary osteocyte-enriched fractions was diluted 1:1 in co-culture medium.

For neutralizing antibody experiments, CXCL10-neutralizing antibody (20 ng/ml; R&D Systems) or IgG was added to co-cultures of wild-type BMCs with *Men1*-deficient primary osteocyte-enriched fractions in co-culture medium.

The medium was changed every 3 days. After 9 days, cells were stained for TRAP activity.

Sorting of GFP+ primary osteocytes from Men1^{Dmp1Cre} × *Rosa*$^{mT/mG}$ *mice*

Primary osteocyte-enriched fractions were isolated from heterozygous (*Men1* flox/+) or homozygous (*Men1* flox/flox) *Men1*^{Dmp1Cre}; Rosa$^{mT/mG}$ mice, and GFP+ cells were sorted

using a FACS LSR II and FACSAria II (BD Biosciences, San Jose, USA). GFP+ cells were used for RNA isolation (see below).

Calvarium ex vivo *culture*

Bone explants from calvaria were obtained by dissecting parietal bones and frontal bones from 7-day-old *Men1*^{flox} or *Men1*^{Dmp1Cre} mice. Calvaria were cut through the sagittal suture and each half was cultured in one well of a 24-well plate. Calvarial halves were treated with 1 µM indomethacin (Sigma-Aldrich) in alpha-MEM supplemented with fatty acid-free serum albumin (Sigma-Aldrich). After overnight incubation, calvarial halves were treated with CXCL10-neutralizing antibody (20 ng/ml) or IgG in alpha-MEM supplemented with 10 nM 1,25-dihydroxyvitamin D3 for 5 days. Medium was collected for CTX ELISA and calvarial halves were used for RNA isolation or histology.

CXCL10-neutralizing antibody injection in vivo

Mouse CXCL10 neutralizing antibody (R&D Systems) or IgG (R&D Systems) was delivered in the space between the subcutaneous tissue and the periosteum over the sagittal suture of the skull. Prior to the injection, all animals were anesthetized. One dose of CXCL10 neutralizing antibody (100 µg/mouse) or IgG (100 µg/mouse), each in a 100 µl volume, was injected subcutaneously. Mice were sacrificed 5 days after the injection.

RNA isolation and QRT-PCR

Calvaria from 3-day-old mice were immersed in RLT buffer (Qiagen, Hilden, Germany) and subsequently homogenized using a Precellys 24 homogenizer (Peqlab, Erlangen, Germany). After centrifugation for 3 min at 10,000g at 4 degree, the supernatant was used for total RNA isolation using RNeasy kit according to the manufacturer's instructions (Qiagen).

Primary osteoblasts or primary osteocyte-enriched fractions were lysed and total RNA was isolated (Qiagen). RNA was reverse-transcribed by a cDNA kit (Applied Biosystems, Carlsbad, USA) and real-time qPCR was performed as previously described [30]. Primer sequences are available upon request.

Immunoblotting

Primary osteoblasts stimulated with either hBMP2 (100 ng/ml) or hTGFβ1 (1 ng/ml; PeproTech) following starvation in serum-free medium for 6 hours were lysed in lysis buffer (Cell Signaling Technology, Danvers, USA) containing proteinase inhibitor (Roche). Vertebra samples (L5) were pulverized using a mortar and pestle, and suspended in the lysis buffer. Total protein amount was determined by BCA assay (Pierce, Waltham, USA) and immunoblotting was performed with antibodies against pSmad1/5/8 (1:1,000; #9511s, Cell Signaling Technology), Smad1/5/8 (1:1,000; sc-6031, Santa Cruz Biotechnology, Dalas, USA), pSmad3 (1:1,000; #9520, Cell Signaling Technology), Smad3 (1:1,000; #9523, Cell Signaling Technology), menin (1:10,000; A300-105A, Bethyl Laboratories, Montgomery, USA), and β-actin (1:1,000; sc-1616, Santa Cruz Biotechnology). Band intensity was analyzed using ImageJ software.

ELISA

Blood was collected into heparin-coated tubes (Sarstedt, Nümbrecht, Germany) and kept at room temperature for at least 30 min before centrifugation at 2,000g at room temperature for 10 min and isolation of the supernatant. PINP ELISA (Immunodiagnostic Systems) were performed with serum, and CTX ELISA (Immunodiagnostic Systems) was performed with serum and conditioned medium, according to the instructions of the manufacturers.

Immunohistochemistry

Paraffin sections (5-µm thickness) of decalcified femurs were subjected to immunohistochemistry as previously described [31]. Antigen retrieval was performed with antigen unmasking solution (Vector Laboratories, Peterborough, UK) at 95 ℃ for 10 min and immunohistochemistry was performed with an antibody against menin (1:3,000; A300-105A, Bethyl Laboratories) and a biotinylated secondary antibody (1:200; Vector Laboratories).

Immunofluorescent staining

Cryosections (100-µm thickness) of decalcified femurs were subjected to immunohistochemistry as described elsewhere [32]. Osteocalcin antibody (1:1,000; LifeSpan BioSciences, Inc., Seattle, USA) and Alexa Fluor 647 secondary antibody (1:200; Thermo Fisher Scientific, Waltham, USA) were used. A Leica TCS-SP8 confocal microscope with an inverted stand (DMi8) and LAS X Software were used for imaging. Images were taken at 40-fold magnification using a Leica HCX PL FL L 40x/0.60 objective.

Men1 *overexpression in MLO-Y4 osteocyte cell line*

RIKEN full-length *Mus musculus Men1* cDNA (F630025E01, Source BioScience, Nottingham, UK) was cloned into a modified lentiviral pLVX-IRES-GFP vector (Clontech Laboratories, Mountain View, USA). The pLVX-IRES-*Men1*-GFP vector or pLVX-IRES-empty-GFP vector was co-transfected with psPax2 vector and pMD2.G vector into LentiX HEK 293T cells using CalPhos™ Mammalian Transfection Kit (Clontech Laboratories). Lentiviruses were collected and the MLO-Y4 osteocyte cell line was transduced according to the manufacturer's instructions. Five days after the transduction, the GFP-expressing cells were sorted using a BD FACSARIA III™ (BD Biosciences) and expanded for further experiments.

Microarray analysis

Total RNAs isolated from primary osteocyte-enriched fractions (80 ng) were processed and labeled for array hybridization using the Ambion WT Expression Kit (Life Technologies, Carlsbad, USA) at the Genomics Core Facility, EMBL Heidelberg, Germany. Labeled, fragmented cDNA (Affymetrix GeneChip® WT Terminal Labeling and Controls Kit, Santa Clara, USA) was hybridized to Mouse Gene 2.0 arrays for 16 hours at 45 ℃ (Affymetrix GeneChip® Hybridization, Wash, and Stain Kit). Arrays were washed and stained using the Affymetrix Fluidics Station 450, and scanned using the Hewlett-Packard GeneArray Scanner 3000 7G. Data analysis was performed with software Expression Console (Affymetrix). The estimated probe signals were normalized using the "Robust Multi-array Average" method, and the expression levels of probe sets were generated in log2 scale, which were then assigned to the annotated gene data (41,346 gene data sets). Probe sets with high expression levels (higher than 50% of the total probe signals) were selected (19,854 gene data sets). These were submitted to DAVID Bioinformatics Resources (NIH, USA) and annotated secreted factors were identified (775 gene data sets). Fold change values were defined as $2^{\text{expression levels (KO-WT)}}$. Probe sets with fold change values >1.4 (30 gene data sets) or <0.7 (81 gene data sets) were selected as possible candidate genes for further validation.

Statistical analysis

Data are presented as mean ± standard errors (SEM). Statistical evaluations of two group comparisons were performed using Student's t-test. Statistical evaluations of experiments with more than two groups were performed using one-way analysis of variance (ANOVA).