Supplementary Figure Legends

Fig. S1. Deletion of *Men1* and bone phenotype of *Men1*^{Runx2Cre} mice.

(**A**) Immunohistochemistry of femoral sections from 12-week-old female *Men1*^{flox} and *Men1*^{Runx2Cre} mice confirmed a diminished menin expression in osteoblasts (top, arrowheads) and in osteocytes (arrows). Scale bar: 25µm. (**B**) Fluorescence of Runx2Cre mice crossed with Rosa$^{mT/mG}$ mice displaying red fluorescence in non-recombined cells and green fluorescence in recombined cells indicating osteoblasts (top, arrowheads) and osteocytes (arrows). Scale bar: 25µm. (**C**) X-ray photographs of whole skeletons of 8-, 12-, and 24-week-old *Men1*^{flox} and *Men1*^{Runx2Cre} mice. (**D**) Von Kossa staining of undecalcified sections of lumbar vertebrae of 12-week-old female *Men1*^{flox} and *Men1*^{Runx2Cre} mice. (**E-H**) Histomorphometry of BV/TV (E), Tb.Th. (F), Tb.N. (G), and Tb.Sp. (H) on sections of lumbar vertebrae (n=5). * $p < 0.05$, ** $p < 0.01$. Data are represented as mean ± SEM.

Fig. S2. Bone integrity in *Men1*^{LysMCre} mice.

(**A**, **B**) BMCs of *Men1*^{flox} and *Men1*^{LysMCre} mice were cultured in the presence of M-CSF and RANKL. Osteoclastogenesis was shown by TRAP staining (A), and recombination of the *Men1* flox allele to the *Men1* null allele was determined at indicated time points by PCR using genomic DNA (B) Tail genomic DNA from homozygous *Men1*^{flox} mice (fl/fl) and heterozygous *Men1*$^{flox/null}$ mice (fl/null) served as controls. (**C-F**) Cancellous parameters from distal femurs of 12-week-old female

Men1^{flox} and *Men1*^{LysMCre} mice were measured by micro CT as described above (n=5). Data are represented as mean ± SEM.

Fig. S3. *In vitro* and *in vivo* function of *Men1* in osteoblasts.

(**A-D**) mRNA levels of *Alpl* (A), *Col1a1* (B), *Runx2* (C), and *Bglap* (D) in calvarial bone of 3-day-old *Men1*^{flox} and *Men1*^{Runx2Cre} mice were determined by QRT-PCR (n=5). (**E-G**) Osteoblast numbers per bone perimeter (N.Ob/B.Pm) (E), osteoblast surface per bone surface (Ob.S/BS) (F), and osteocyte numbers per bone area (N.Ot/B.Ar) (G) in trabecular bone of lumbar vertebral sections of 12-week-old female *Men1*^{flox} and *Men1*^{Runx2Cre} mice were measured by histomorphometry (n=4 or 5). ** $p < 0.01$. (**H**) Primary calvarial osteoblasts were isolated from *Men1*^{flox} and *Men1*^{gtRosaCreERT2} mice. After pretreatment with 4-OHT to allow recombination of the *Men1*flox allele, cells were seeded for preconfluencial cell number up to 7 days (n=3). (**I-L**) Adipose tissue-derived mesenchymal stromal cells (I, J) and bone marrow stromal cells (K, L) were isolated from *Men1*^{flox} and *Men1*^{gtRosaCreERT2} mice. After pretreatment with 4-OHT to allow recombination of the *Men1*-floxed allele, cells were cultured with or without osteogenic induction (OI) medium in the absence or presence of BMP2 for 7 and 12 days and stained for alkaline phosphatase (I, K) and alizarin red (J, L), respectively. (**M-O**) mRNA expression levels of *Alpl* (M), *Sp7* (N), and *Men1* (O) were measured by QRT-PCR in the cells treated for 7 days as described in (I) (n=3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. Untreated *Men1*^{flox}. (**P**) Expression levels of phosphorylated Smad1/5/8, Smad1/5/8, and menin in 4-OHT-treated primary osteoblasts following BMP2 stimulation for the indicated times were determined by immunoblotting. (**Q**, **R**) Induced mRNA levels of the BMP2 target

genes *Id1* (Q) and *Dlx5* (R) in 4-OHT-treated primary osteoblasts following BMP2 exposure for the indicated times were determined by QRT-PCR (n=3). (**S**) Expression levels of phosphorylated Smad3, Smad3, and menin in 4-OHT-treated primary osteoblasts following TGFβ1 stimulation for the indicated times were determined by immunoblotting. (**T**, **U**) Induced mRNA levels of the TGFβ target genes *Serpine1* (T) and *Skil* (U) in 4-OHT-treated primary osteoblasts following TGFβ exposure for the indicated times were determined by QRT-PCR (n=3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. $Men1^{flox}$ 0h treatment. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ vs. $Men1^{gtRosaCreERT2}$ 0h treatment. (**V**, **W**) Fluorescent micrographs of dual calcein labeling (V) and its quantitative analysis of bone formation rate per bone surface (BFR/BS) (W) by dynamic histomorphometry in lumbar vertebral sections of 12-week-old female $Men1^{flox}$ and $Men1^{Runx2Cre}$ mice (n=4 or 5). (**X**, **Y**) Same as (V, W) except in femurs of 12-month-old female $Men1^{flox}$ and $Men1^{Runx2Cre}$ mice (n=4 or 5). ** $p < 0.01$. Data are represented as mean ± SEM.

Fig. S4. Osteocyte-osteoclast crosstalk.

(**A**, **B**) Osteoclast numbers per bone perimeter (N.Oc/B.Pm) (A) and osteoclast surface per bone surface (Oc.S/BS) (B) in trabecular bone of lumbar vertebral sections were measured by histomorphometry in $Men1^{Runx2Cre}$ mice (n=4 or 5). (**C-E**) TRAP staining of calvaria (C), number of osteoclasts (D), and osteoclast surface (E) in 12-week-old female $Men1^{Runx2Cre}$ mice. . Scale bar: 50μm. (**F**) Micro CT reconstruction of calvaria of 12-week-old $Men1^{flox}$ and $Men1^{Runx2Cre}$ mice displaying enhanced porosity in mutant calvaria. ** $p < 0.01$. Data are represented as mean ± SEM.

Fig. S5. *Men1* loss in *Men1*^{gtRosaCreERT2}, *Men1*^{Dmp1Cre}, and Rosa$^{mT/mG}$ × *Men1*^{Dmp1Cre} mice.

(**A**, **B**) QRT-PCR determination of mRNA levels of *Alpl* (A) and *Dmp1* (B) in isolated osteocyte-enriched (Ot) fraction and osteoblast (Ob) fraction. (**C**) mRNA expression of *Men1* was determined by QRT-PCR in 4-OHT-pretreated osteocyte-enriched fractions isolated from *Men1*^{flox} or *Men1*^{gtRosaCreERT2} mice (n=3). (**D**) Immunohistochemistry of menin in femoral sections from 12-week-old female *Men1*^{flox} and *Men1*^{Dmp1Cre} mice. Arrows indicate osteocytes. Scale bar: 100μm. (**E**) Histograms showing FACS-sorted GFP-positive cells of *Men1*^{Dmp1Cre} mice crossed to Rosa$^{mT/mG}$ mice (used for the QRT-PCR analysis shown in Fig. 3A). (**F**) Immunofluorescent staining of osteocalcin (red) in *Men1*^{Dmp1Cre}; Rosa$^{mT/mG}$ reporter mice. Osteocalcin-expressing cells (arrowheads) and EGFP indicating Dmp1-expressing cells (arrows) were visualized (n=7). Scale bar: 50μm. (**G**, **H**) Determination of the serum levels of the bone formation markers RANKL (G) and OPG (H) in 12-week-old female *Men1*^{flox} and *Men1*^{Dmp1Cre} mice (n=5). ** $p < 0.01$, *** $p < 0.001$. Data are represented as mean ± SEM.

Fig. S6. Gene expression *in vivo* and functional effect of CXCL10-neutralizing antibody.

(**A**, **B**) QRT-PCR determination of mRNA expression of *Tnfsf11* (A) and *Tnfrsf11b* (B) in calvarial bone of 3-day-old *Men1*^{flox} and *Men1*^{Dmp1Cre} mice (n=3). (**C**-**E**) Osteoclastogenesis was visualized by TRAP staining of osteoclasts

supplemented with conditioned medium from 4-OHT-pretreated *Men1*^{flox} or *Men1*^{gtRosaCreERT2} osteocyte-enriched fractions (C). Number of multinucleated TRAP-positive cells (D) and their area (E) were determined (n=3). Scale bar: 25μm. (**F-P**) mRNA expression levels of *Saa3* (F), *Mmp13* (G), *Hpse* (H), *Cxcl5* (I), *Tgfb2* (J), *Ltbp3* (K), *Bmp5* (L), *Igfbp5* (M), *Bmp6* (N), *Igf2* (O), and *Dkk2* (P) were determined by QRT-PCR in calvarial bone of 3-day-old *Men1*^{flox} and *Men1*^{Dmp1Cre} mice (n=5). (**Q**) QRT-PCR determination of mRNA expression of *Cxcl10* in calvarial bone of 3-day-old *Men1*^{flox} and *Men1*^{Runx2Cre} mice (n=4 or 5). (**R**, **S**) CXCL10-neutralizing antibody (20 ng/ml) or IgG was added to co-cultures of wild-type BMCs with *Men1*^{flox} control osteocyte-enriched fractions treated with 4-OHT. Number of multinucleated TRAP-positive cells (R) and their area (S) were determined (n=3). * $p < 0.05$. Data are represented as mean ± SEM.