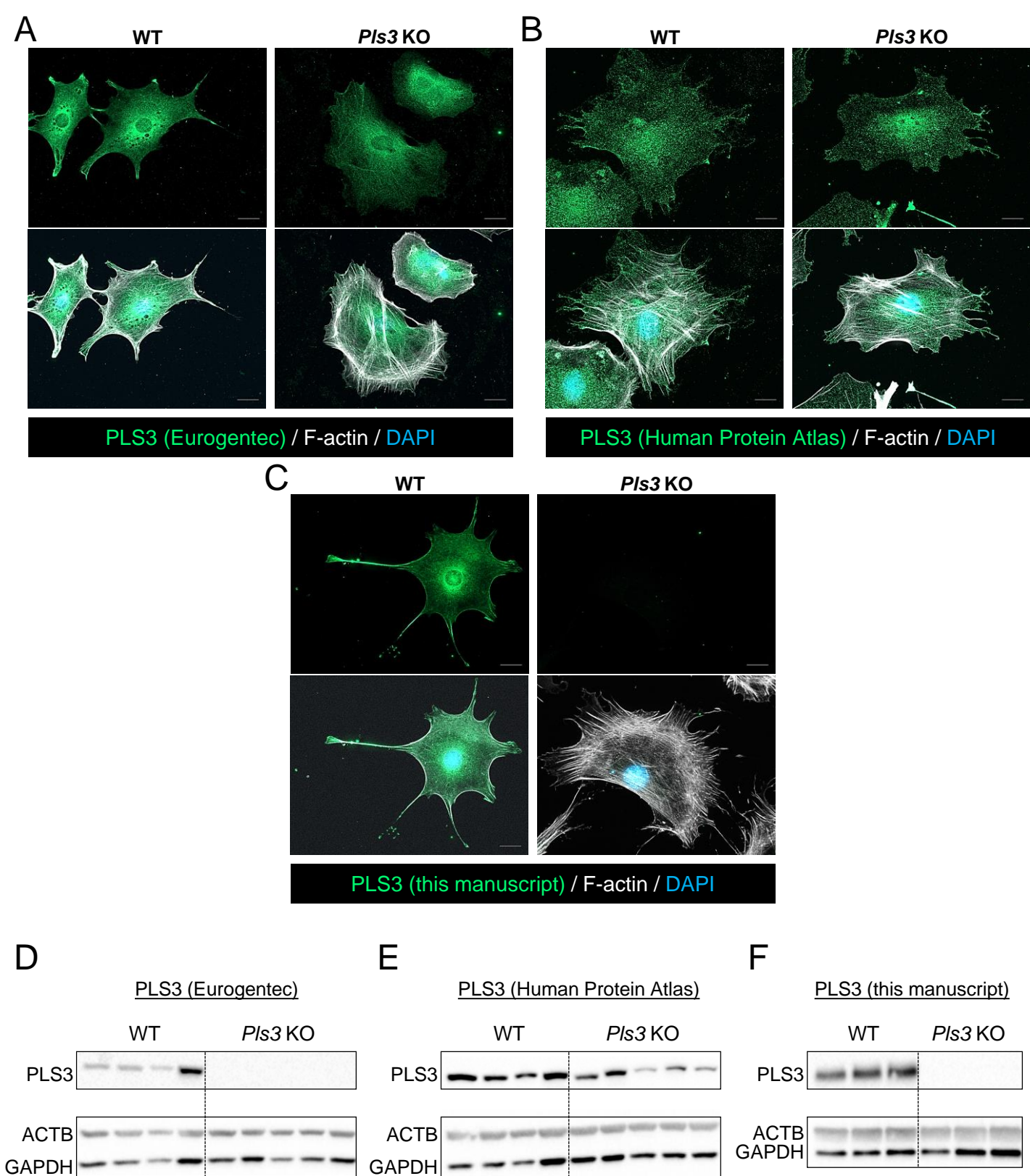


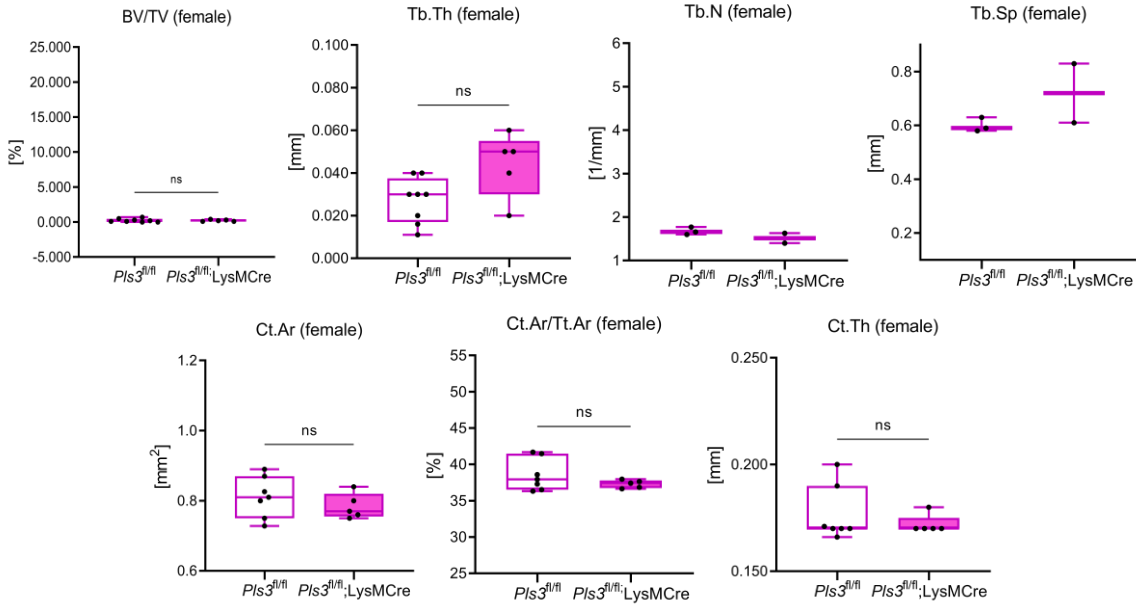
*Supplemental Fig. S1: Generation and characterization of osteoclast-specific *Pls3* KO (*Pls3^{fl/fl}*;LysMCre^{tg/0}) mice. (A) Genotyping of male and female osteoclast-specific *Pls3* KO mice and *Pls3*-floxed mice confirmed the successful removal of the FRT sites resulting in floxed *Pls3* alleles (cassette) (upper parts), and the presence of LysMCre (lower part). A WT sample was used as positive control. (B) Exemplary images of the lateral view of mouse whole body radiographs of 12-week-old female WT, *Pls3^{fl/fl}*, and *Pls3^{fl/fl}*;LysMCre^{tg/0} mice used for the calculation of the kyphosis index. For the kyphosis index measurements and calculations, female and male osteoclast-specific *Pls3* KO mice and *Pls3*-floxed mice were used. (C) PLS3 levels in osteoclasts derived from bone marrow of 12-week-old *Pls3^{fl/fl}* and (D) *Pls3^{fl/fl}*;LysMCre^{tg/0} mouse (lane 1) and from osteoclasts derived from spleen of 4-week-old WT and ubiquitous *Pls3* KO mouse (lane 2 and 3). Exposure 55kV/30seconds. N = 6-10. All results are shown as mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: unpaired two-tailed Student's *t*-test.*



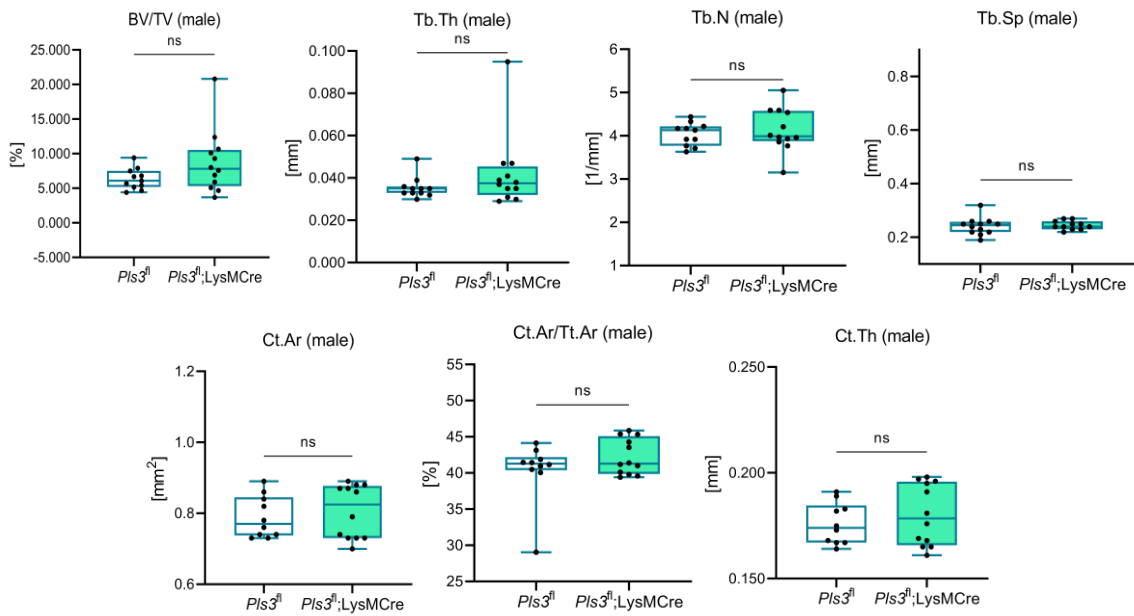
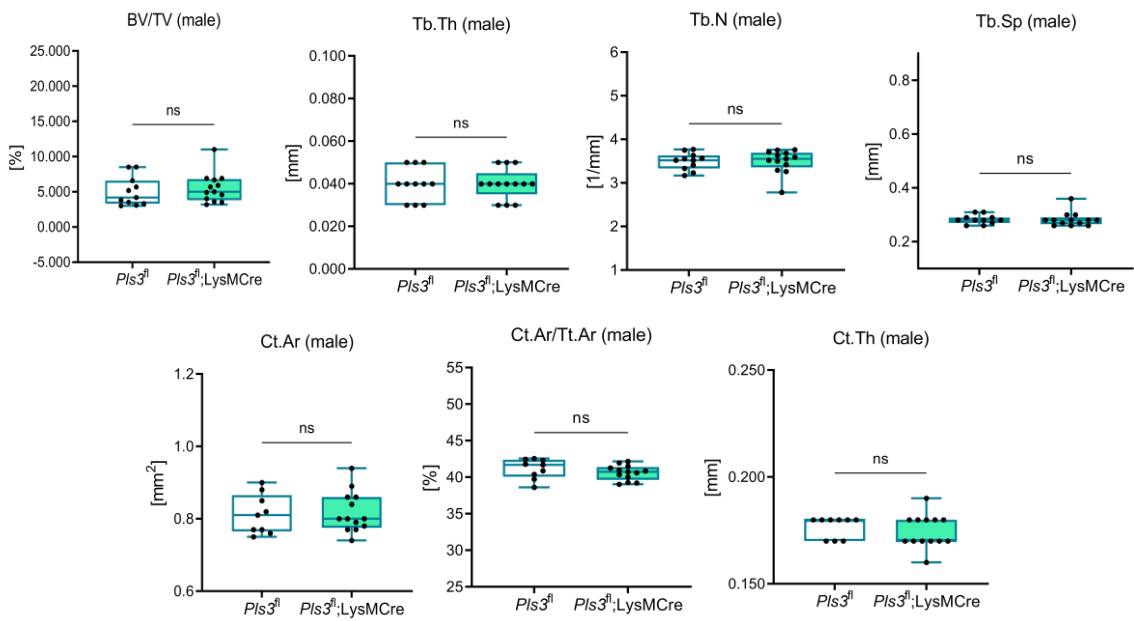
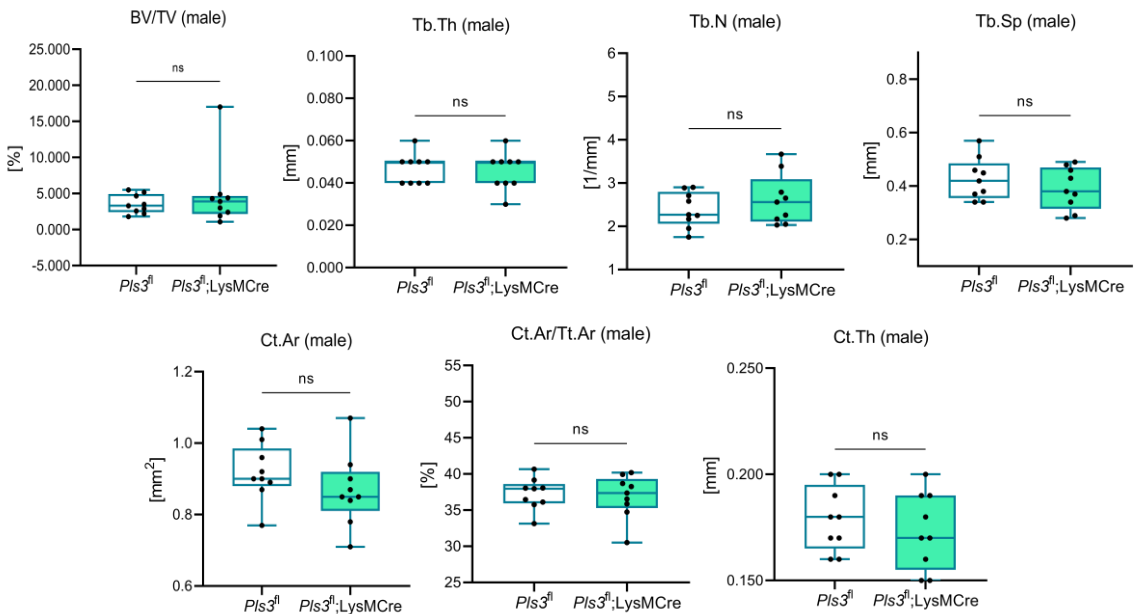
*Supplemental Fig. S2: Determination of the specificity of different PLS3 antibodies on cultivated murine embryonic fibroblasts (MEFs) and osteoclasts from WT and *Pls3* KO mice. (A-C) Immunofluorescence staining of cultivated WT control and *Pls3* KO MEFs using (A) PLS3 antibody from Eurogentec (1238), (B) PLS3 antibody from Human Protein Atlas (HPA020433), and (C) PLS3 antibody developed in this manuscript (3772). PLS3 is shown in green, F-actin in white, and nuclei are stained in blue. (D-F) Western blot analysis of PLS3 using proteins from WT control and *Pls3* KO primary differentiated osteoclasts with (A) PLS3 antibody from Eurogentec (1238), (B) PLS3 antibody from Human Protein Atlas (HPA020433), and (C) PLS3 antibody developed in this manuscript (3772). Scale bar: 20 μ m. (D-F) N=3-5.*

A

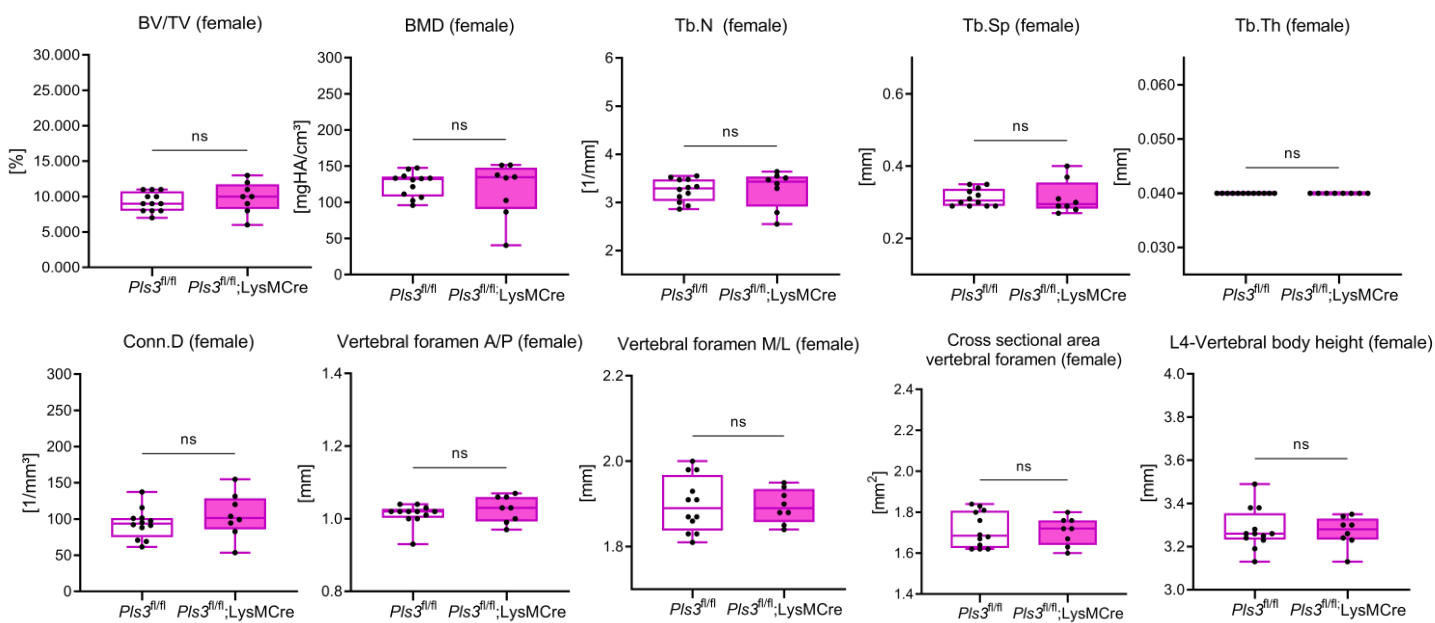
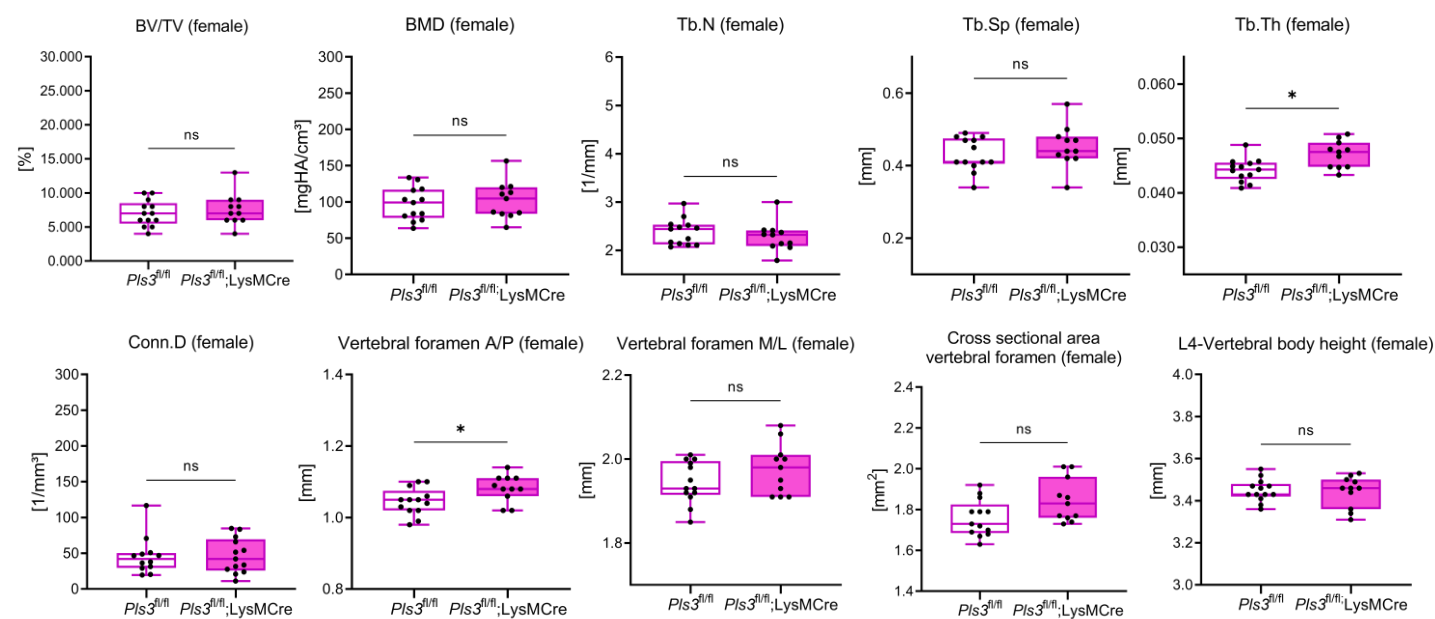
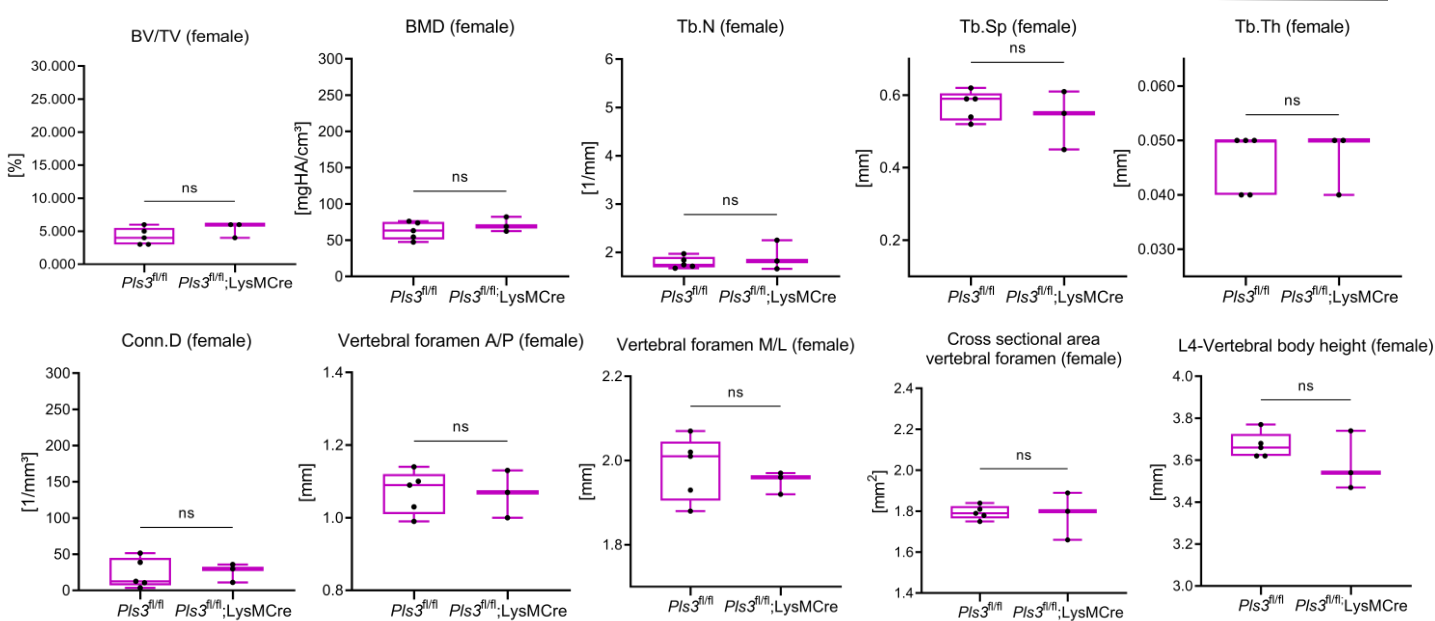
48-weeks



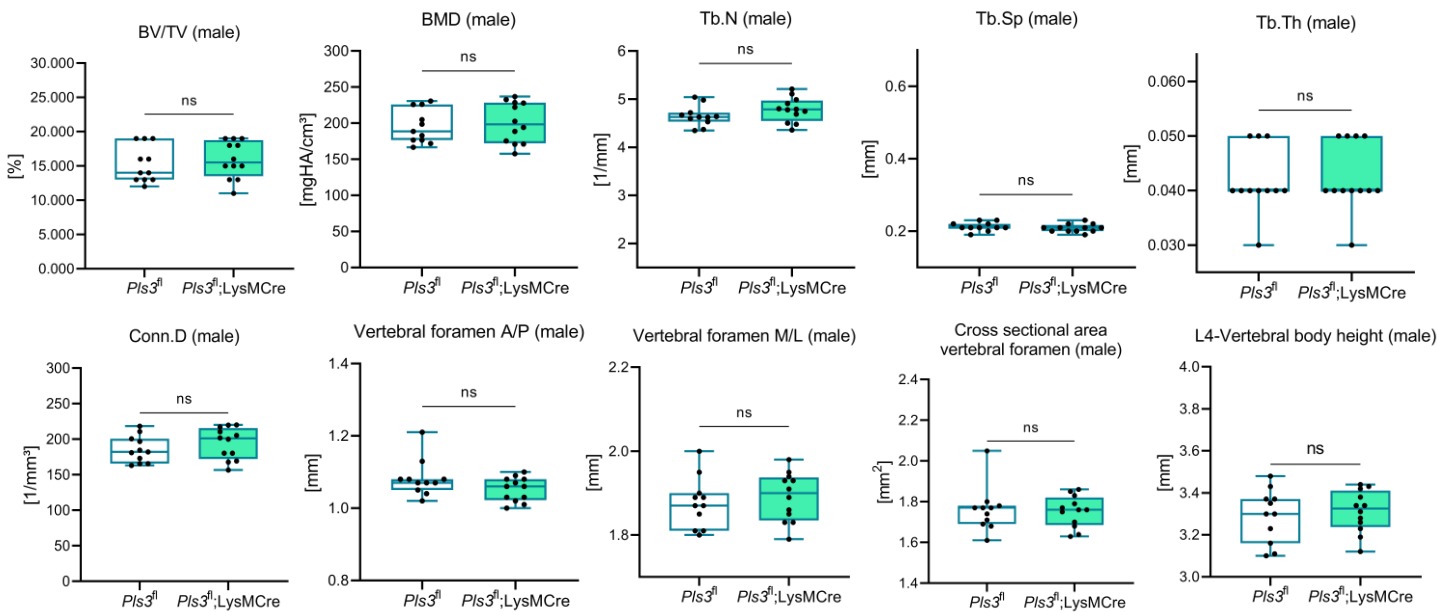
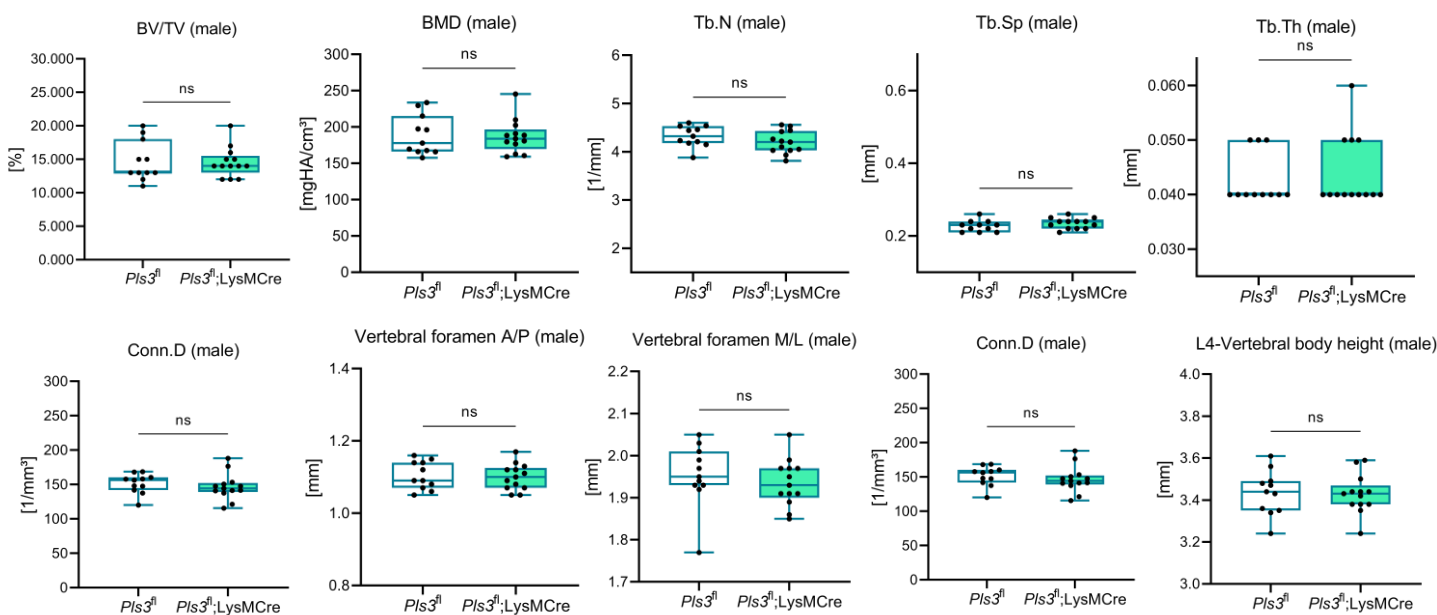
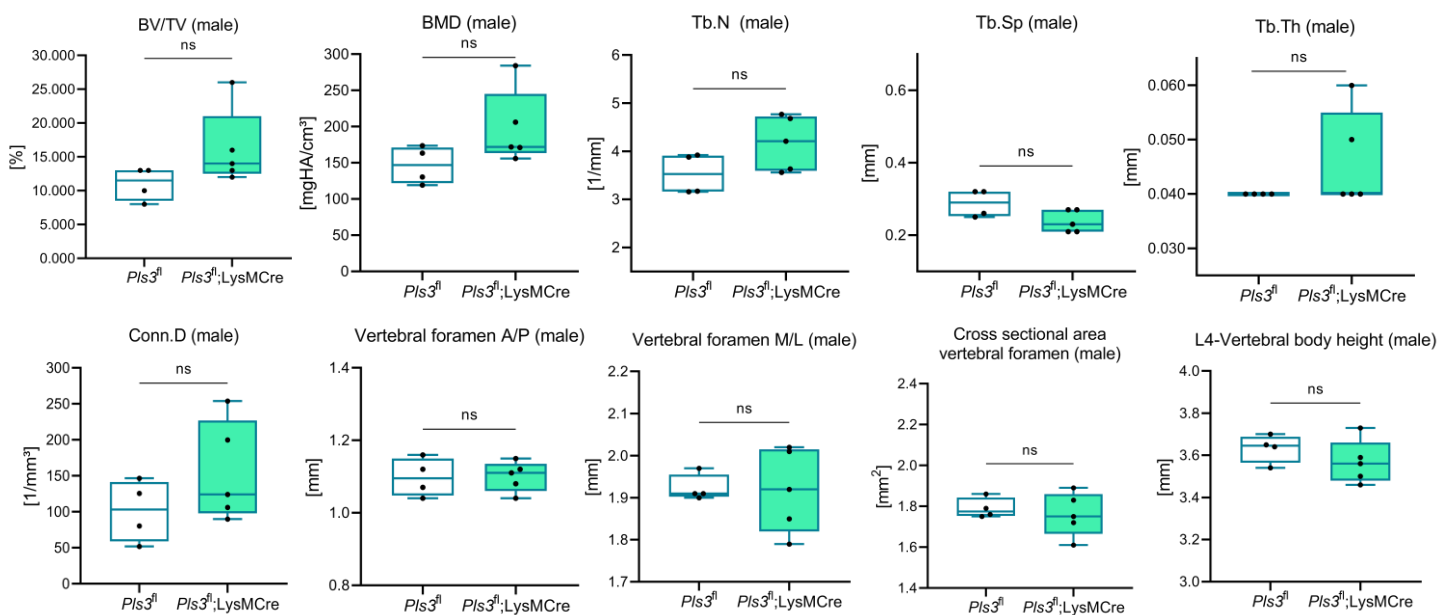
Supplemental Fig. S3: MicroCT data of the femora from 48-week-old female *Pls3^{fl/fl};LysMCre^{tg/0}* mice and their *Pls3^{fl/fl}* littermates. . Shown are bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, mm), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), cortical area (Ct.Ar, mm²), ratio of Ct.Area to tissue area (Ct.Ar/Tt.Ar, %) ratio and cortical thickness (Ct.Th, mm). N = 2-5 of 48-week-old female *Pls3^{fl/fl};LysMCre^{tg/0}* mice; N = 3-8 of 48-week-old female *Pls3^{fl/fl}* mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.

A**12-weeks****B****24-weeks****C****48-weeks**

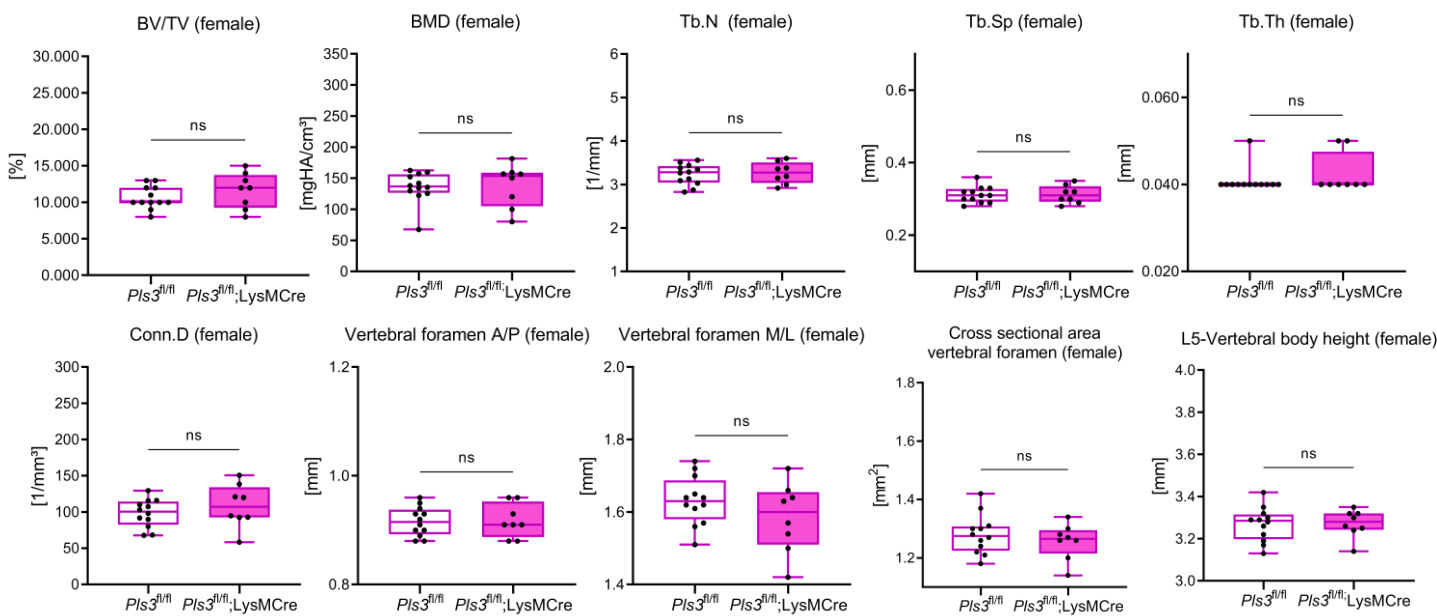
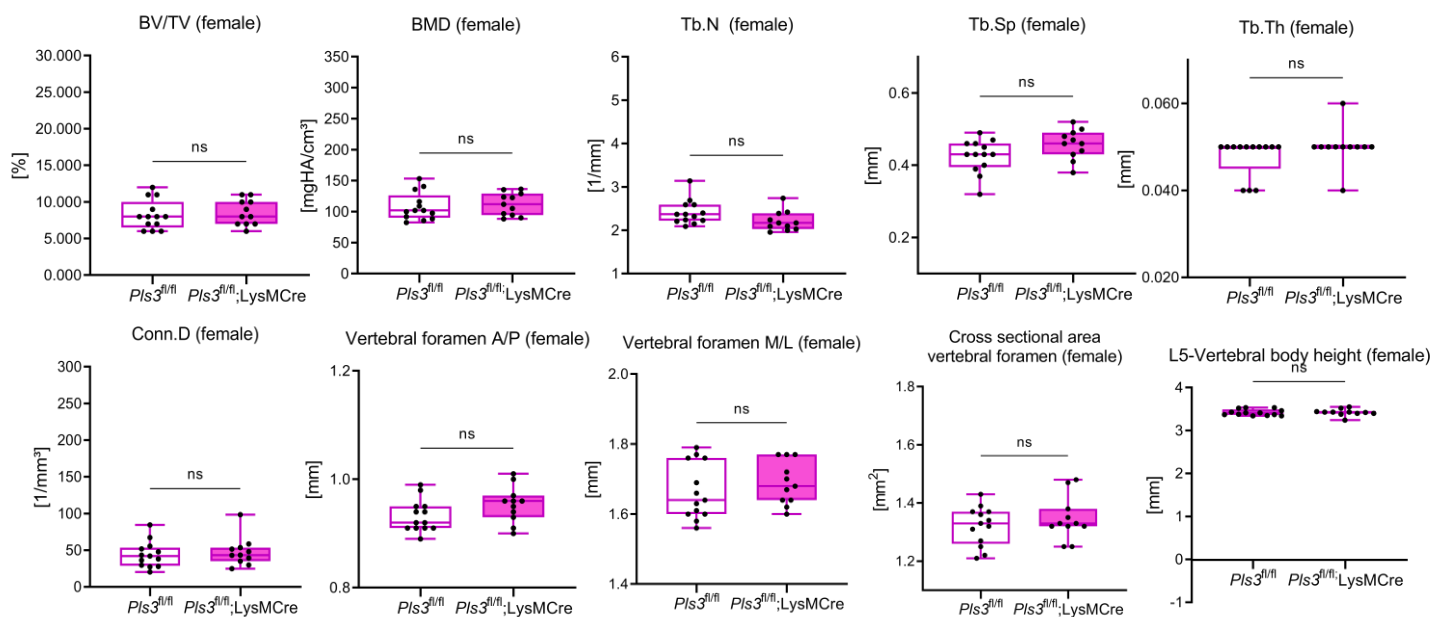
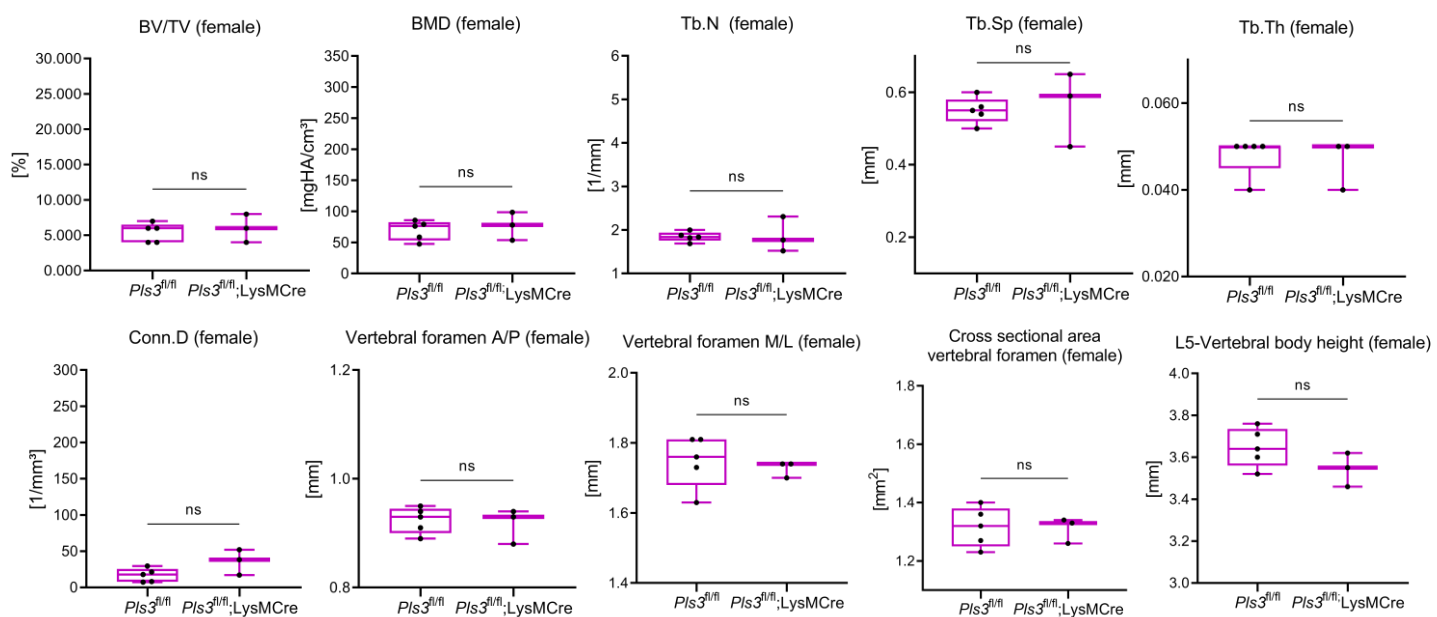
Supplemental Fig. S4: MicroCT data of femora from male $Pls3^{fl};LysMCre^{tg/0}$ mice and their $Pls3^{fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) 24-, and (C) 48-week-old mice. N = 12 of 12-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 12-week-old male $Pls3^{fl}$ mice; N = 13 of 24-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 9-11 of 24-week-old male $Pls3^{fl}$ mice; N = 9 of 48-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 9 of 48-week-old male $Pls3^{fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.

A**12-weeks****B****24-weeks****C****48-weeks**

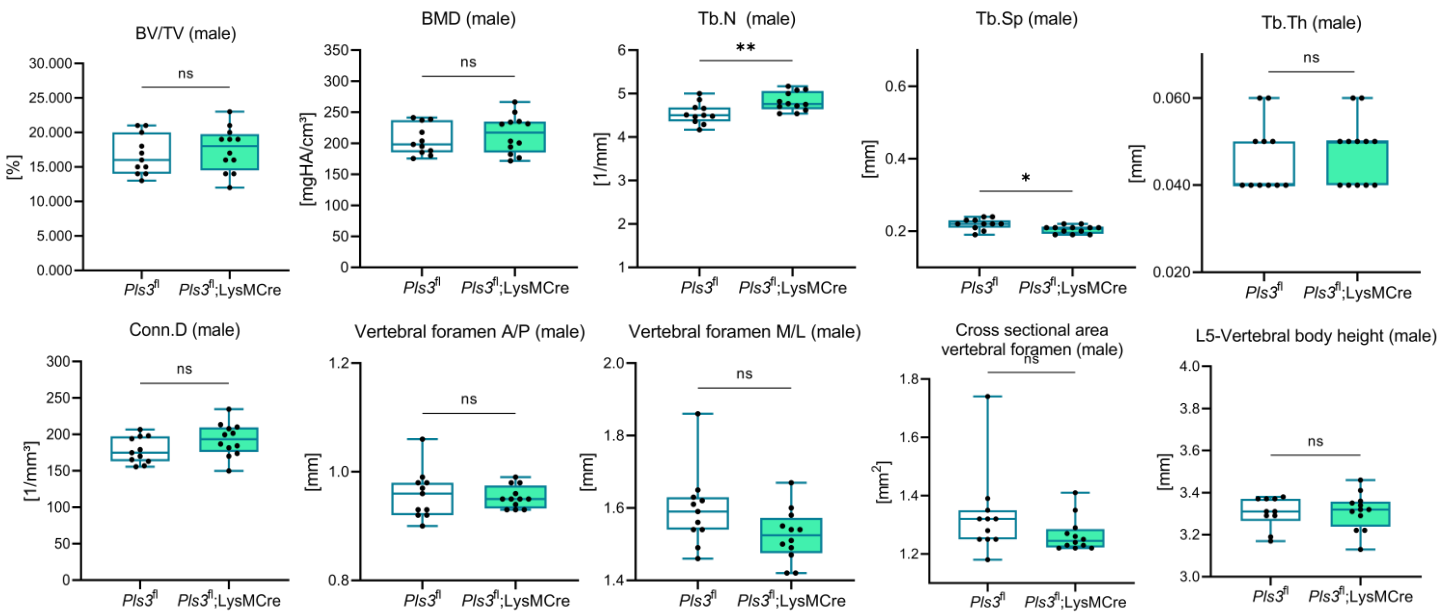
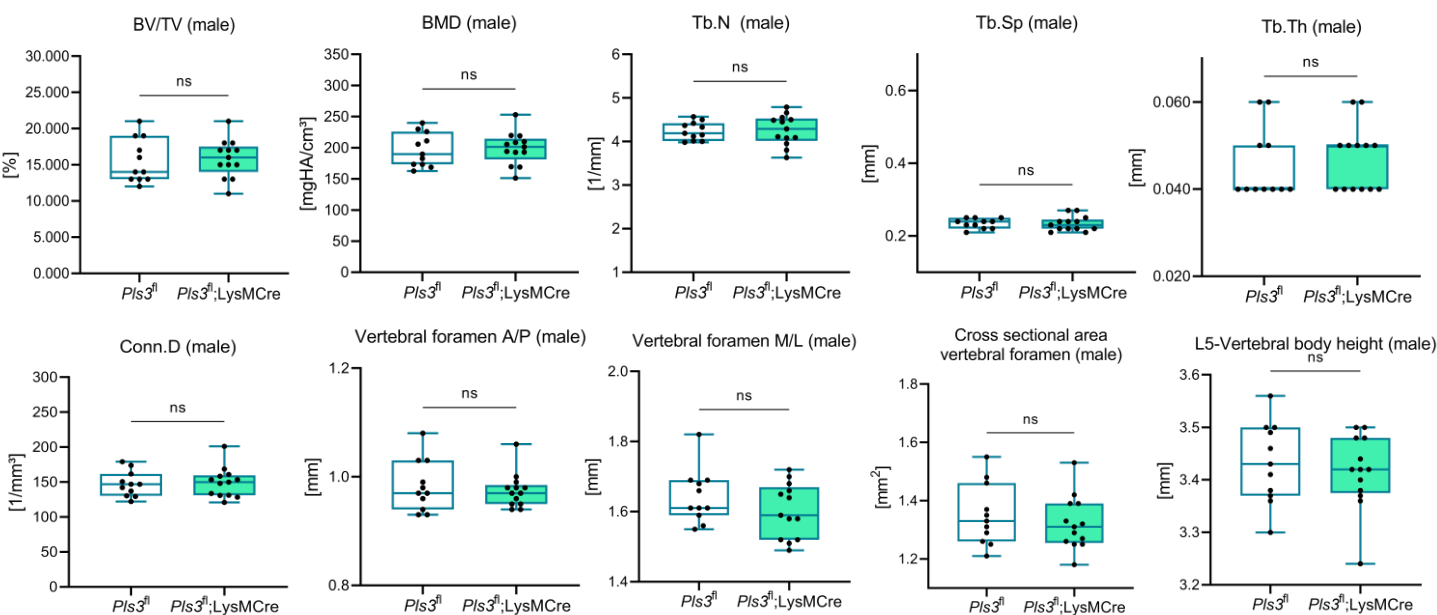
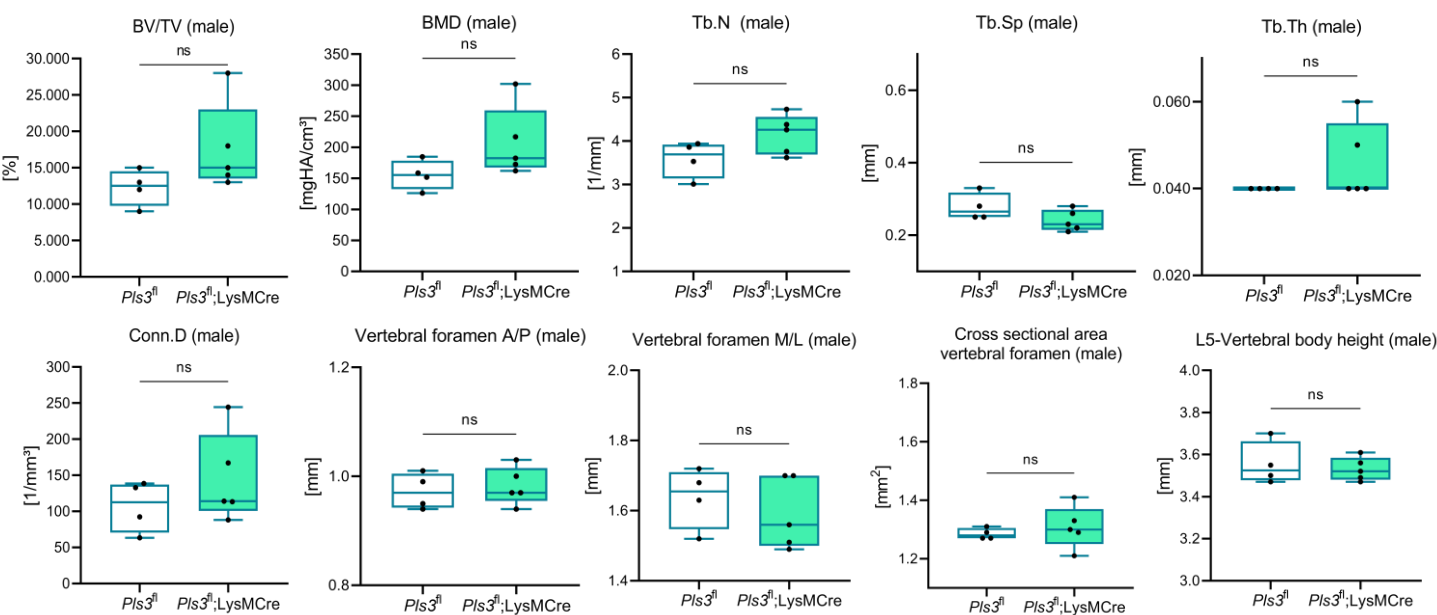
*Supplemental Fig. S5: MicroCT data of spine L4 from female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl/fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) for 24-, and (C) 48-week-old female mice. N = 8 of 12-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 12 of 12-week-old female $Pls3^{fl/fl}$ mice; N = 11 of 24-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 13 of 24-week-old female $Pls3^{fl/fl}$ mice; N = 3 of 48-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 5 of 48-week-old female $Pls3^{fl/fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.*

A**12-weeks****B****24-weeks****C****48-weeks**

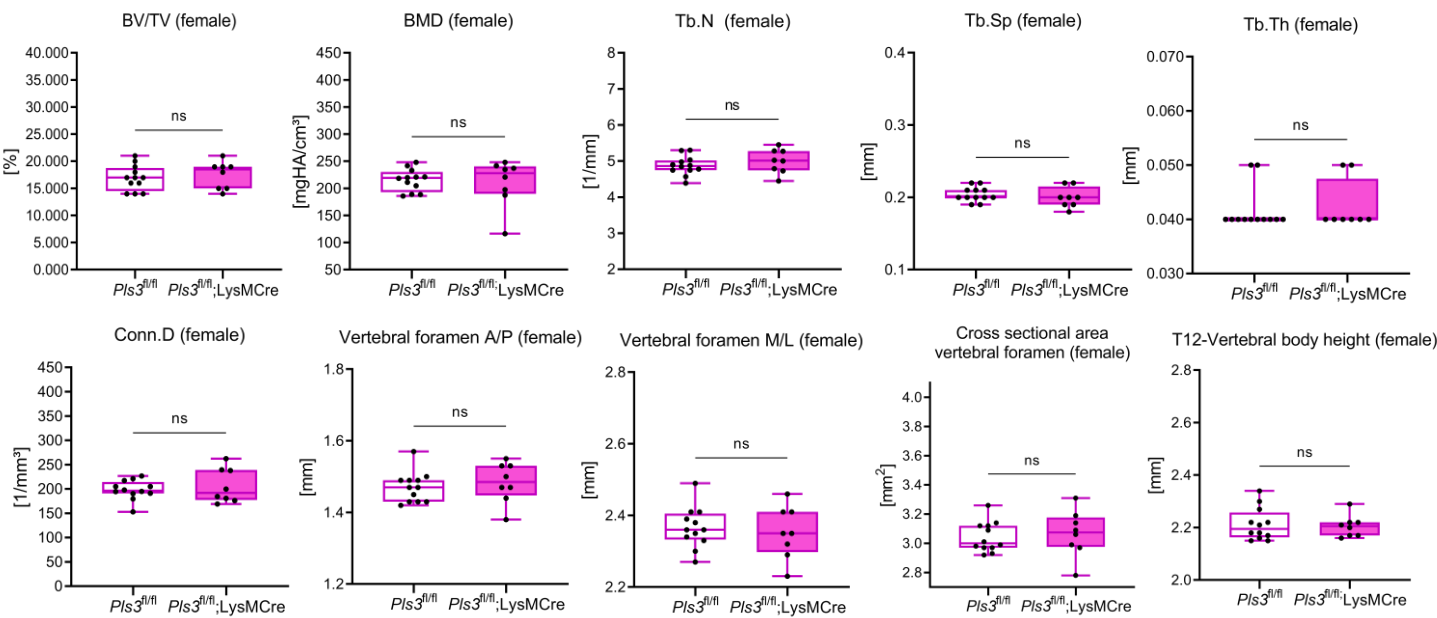
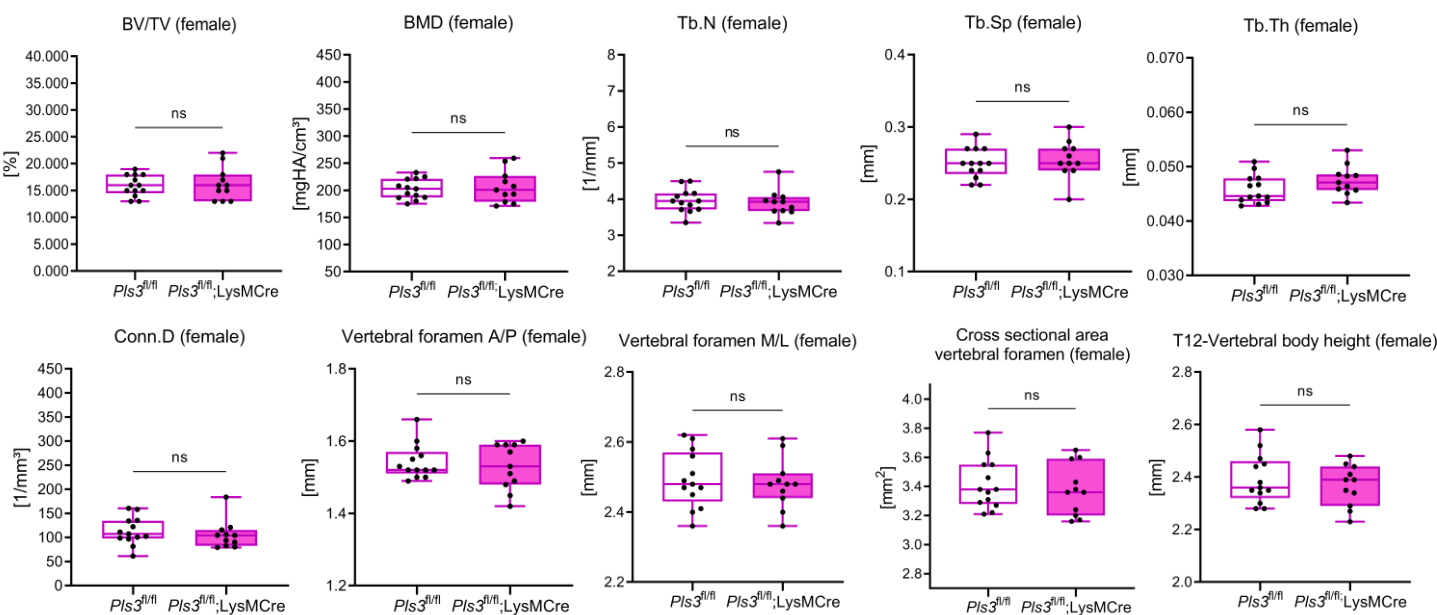
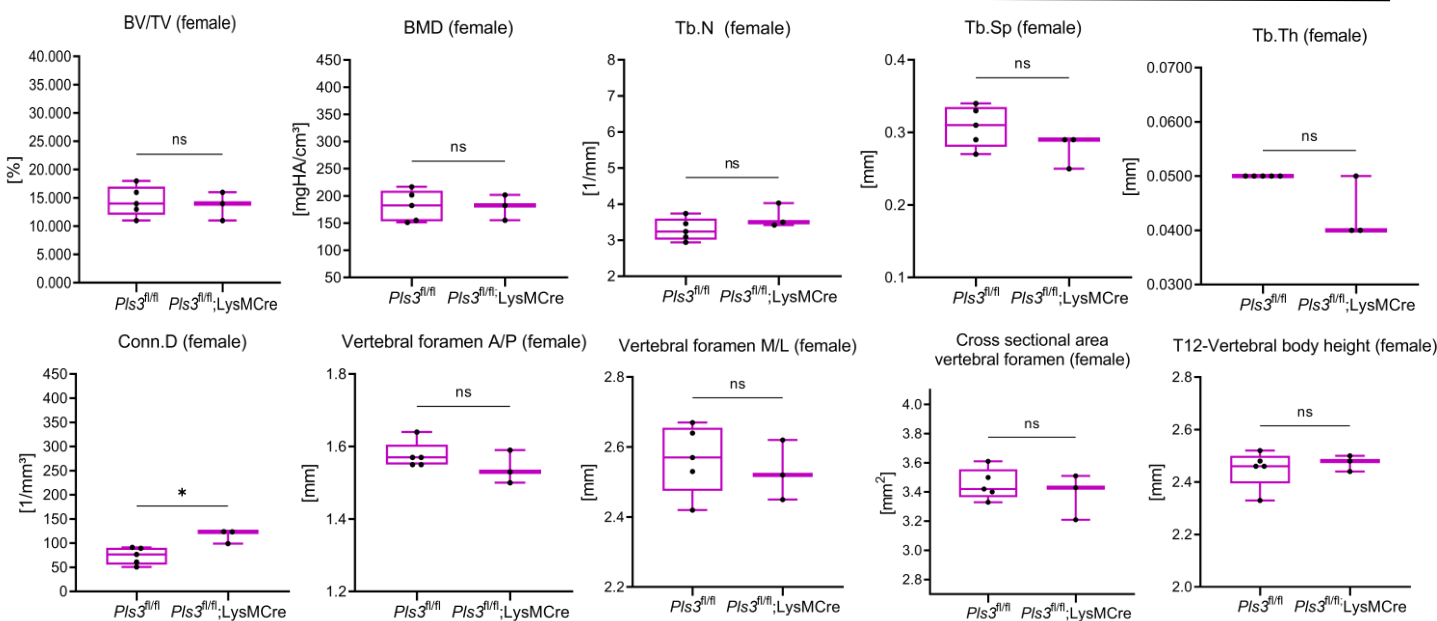
Supplemental Fig. S6: MicroCT data of spine L4 from male $Pls3^{fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) for 24-, and (C) 48-week-old male mice. N = 12 of 12-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 12-week-old male $Pls3^{fl}$ mice; N = 13 of 24-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 24-week-old male $Pls3^{fl}$ mice; N = 5 of 48-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 4 of 48-week-old male $Pls3^{fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.

A**12-weeks****B****24-weeks****C****48-weeks**

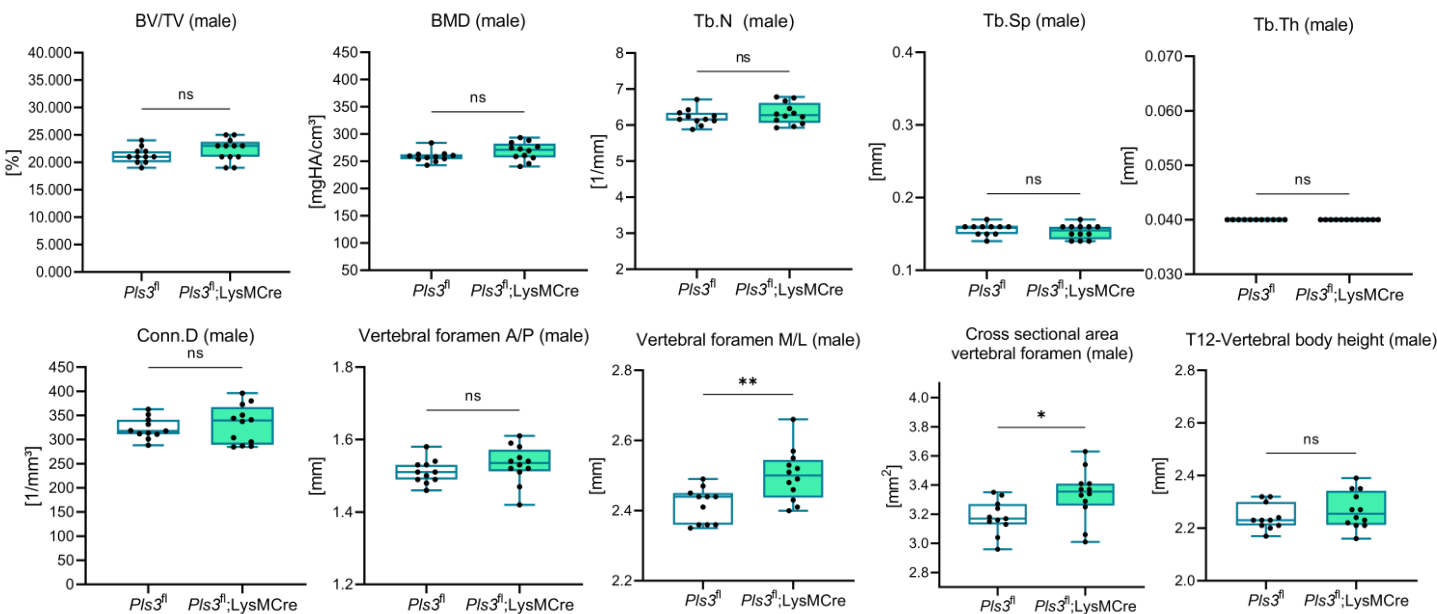
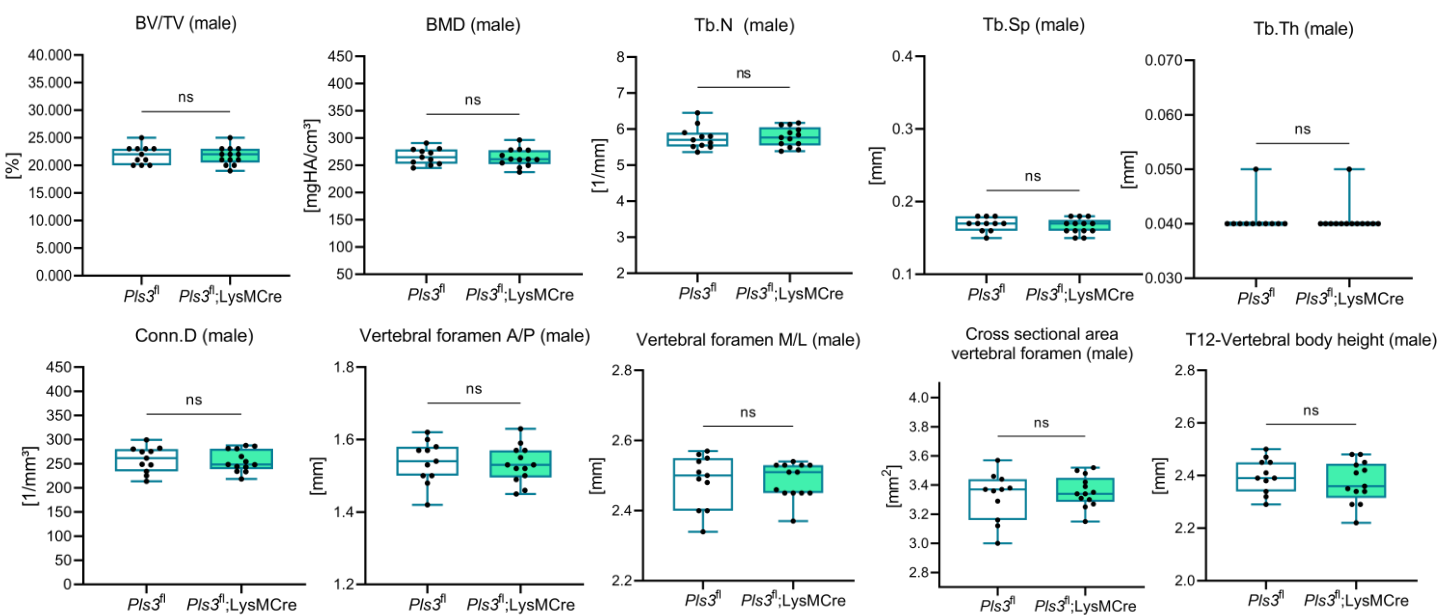
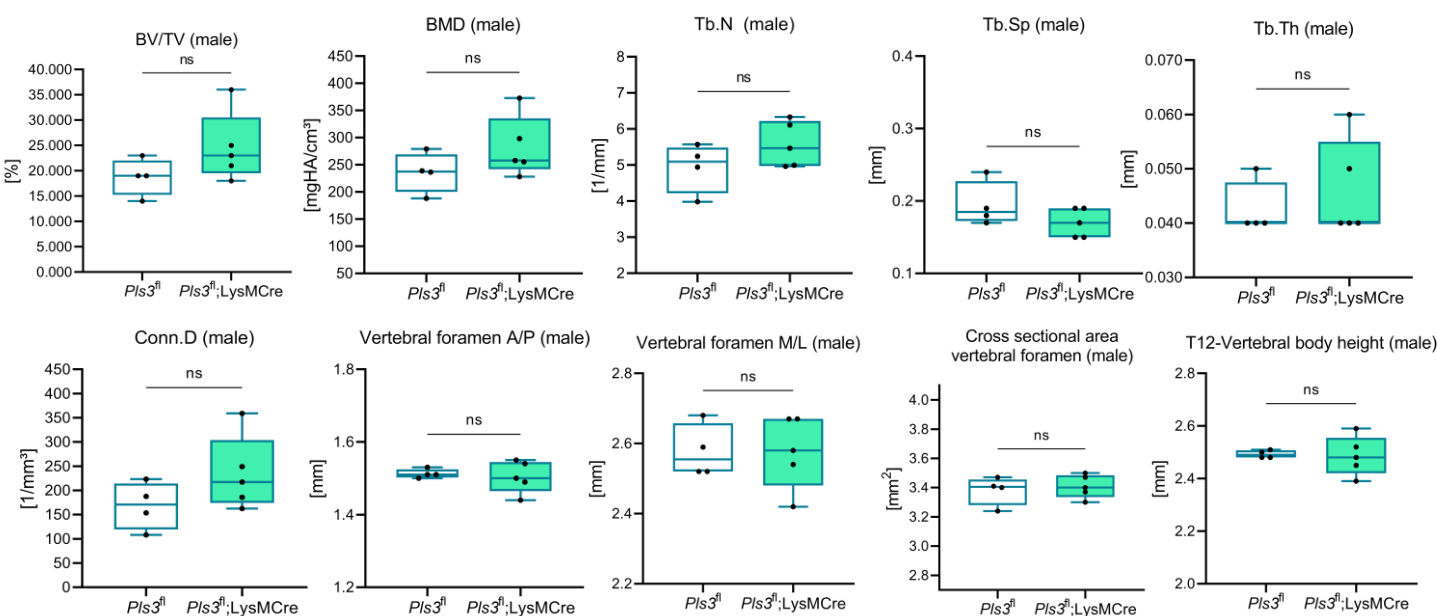
*Supplemental Fig. S7: MicroCT data of spine L5 from female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl/fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) for 24-, and (C) 48-week-old female mice. N = 8 of 12-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 12 of 12-week-old female $Pls3^{fl/fl}$ mice; N = 11 of 24-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 13 of 24-week-old female $Pls3^{fl/fl}$ mice; N = 3 of 48-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 5 of 48-week-old female $Pls3^{fl/fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.*

A**12-weeks****B****24-weeks****C****48-weeks**

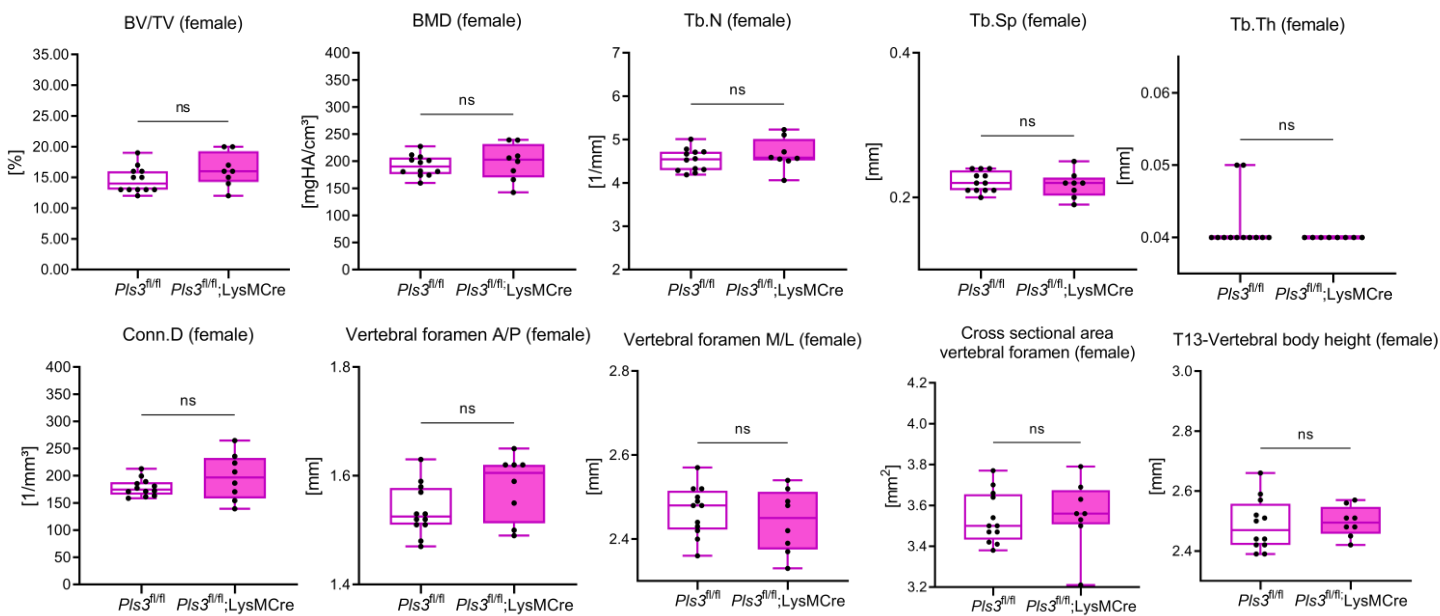
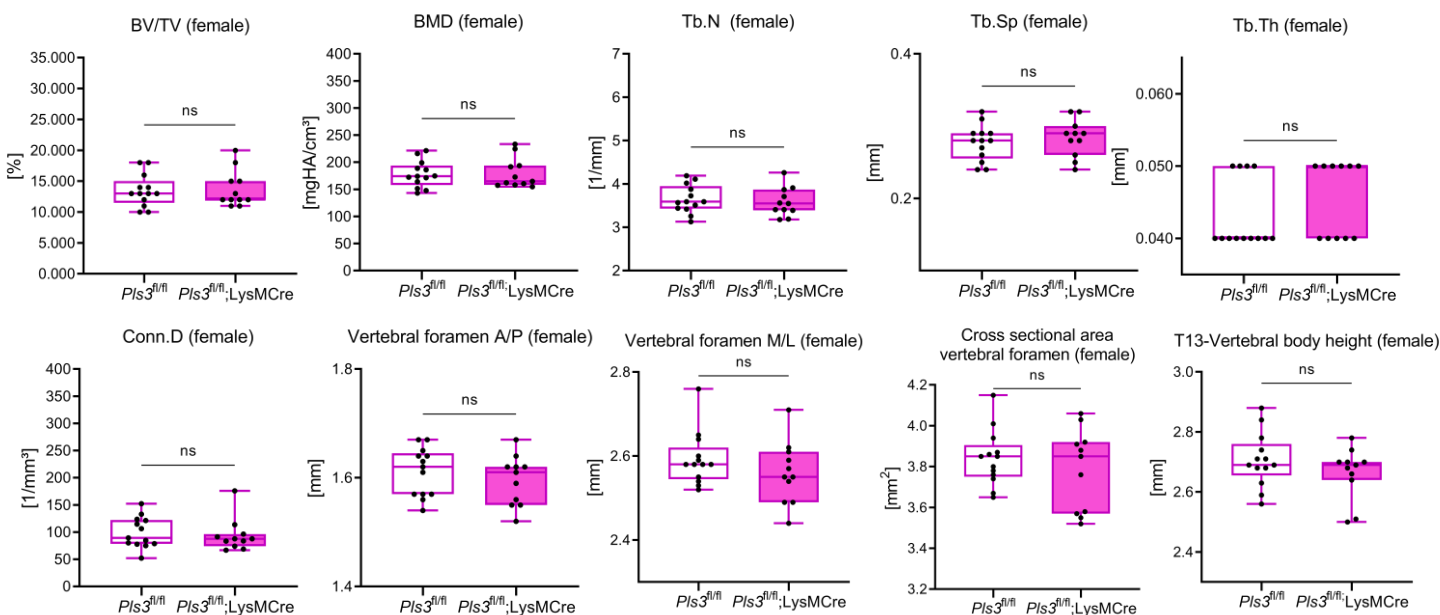
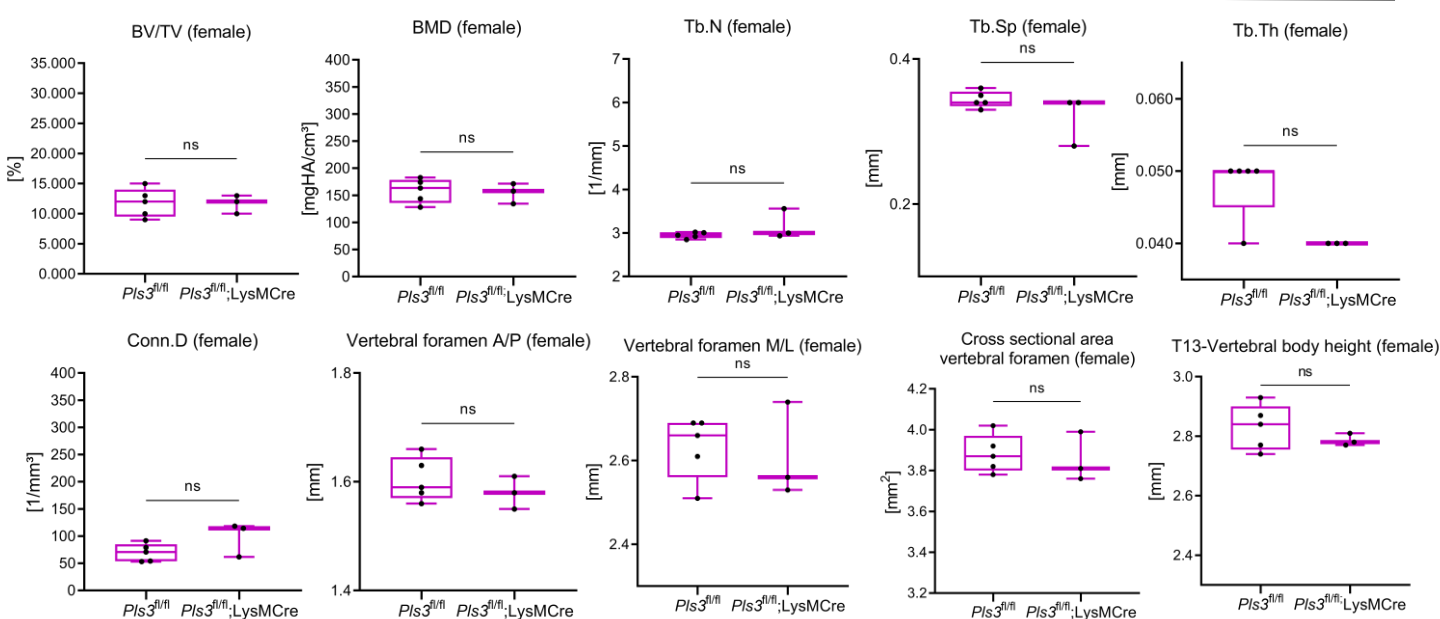
Supplemental Fig. S8: MicroCT data of spine L5 from male $Pls3^{fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) for 24-, and (C) 48-week-old male mice. N = 12 of 12-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 12-week-old male $Pls3^{fl}$ mice; N = 13 of 24-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 24-week-old male $Pls3^{fl}$ mice; N = 5 of 48-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 4 of 48-week-old male $Pls3^{fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.

A**12-weeks****B****24-weeks****C****48-weeks**

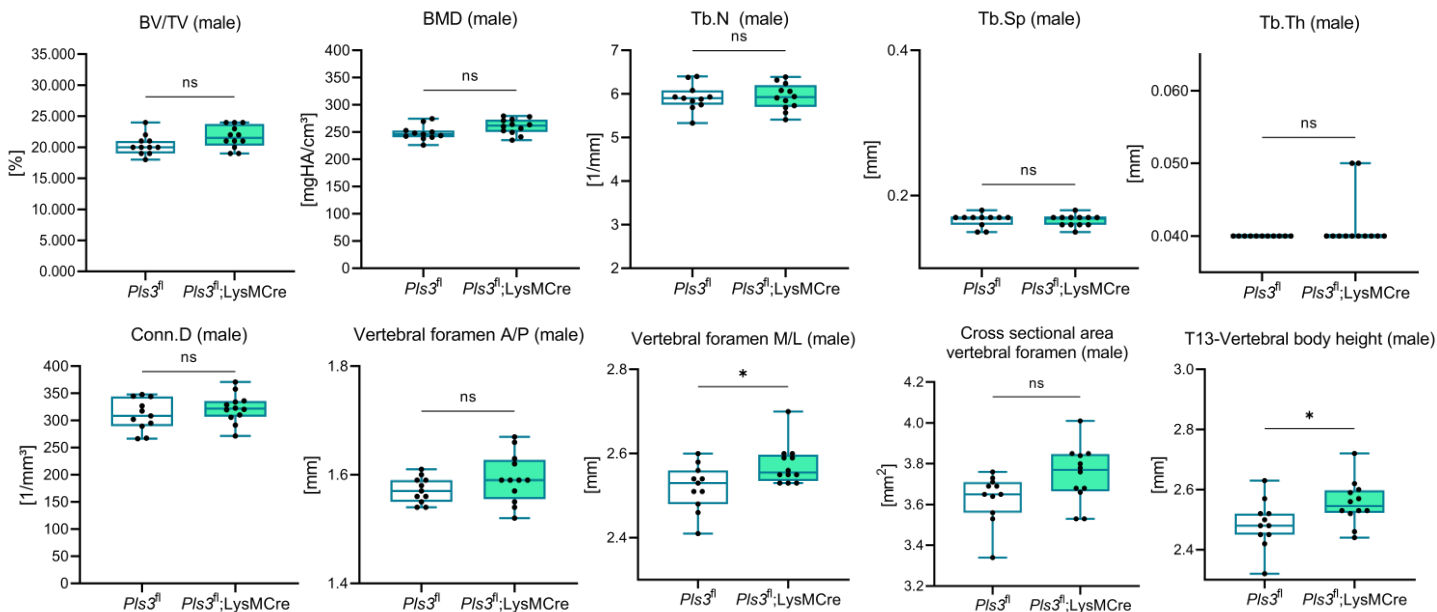
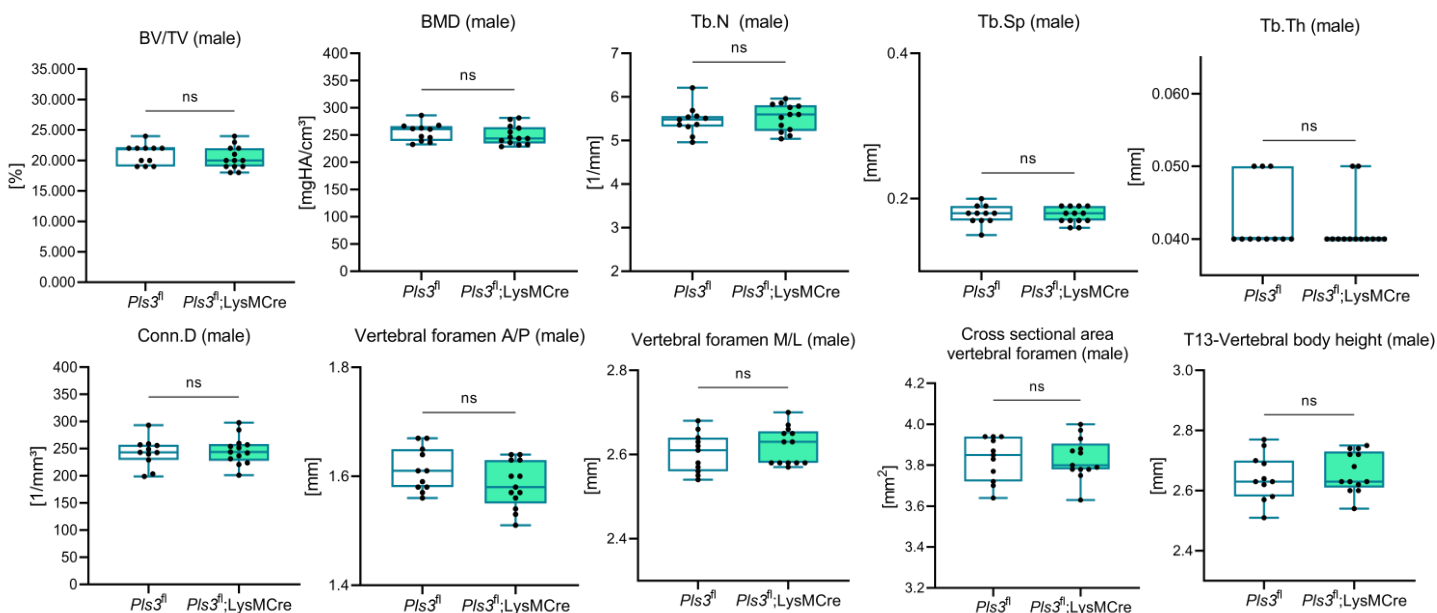
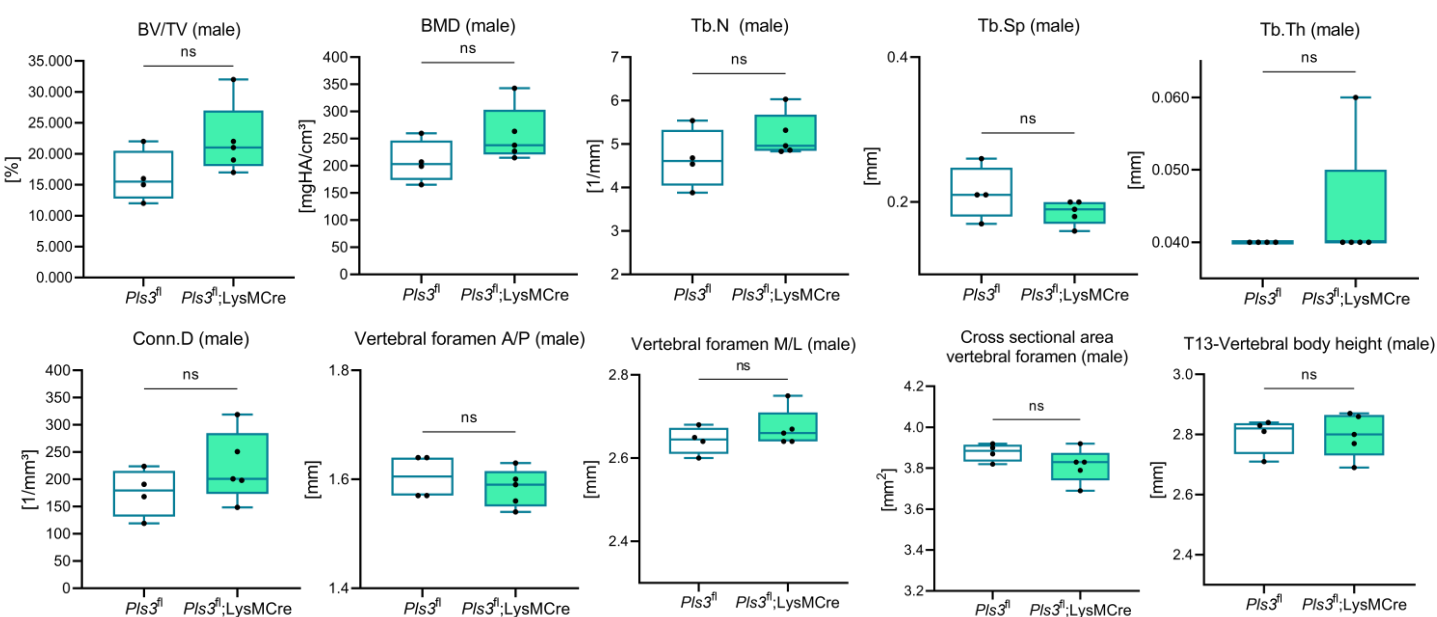
Supplemental Fig. S9: MicroCT data of spine T12 from female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl/fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) for 24-, and (C) 48-week-old female mice. N = 8 of 12-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 12 of 12-week-old female $Pls3^{fl/fl}$ mice; N = 11 of 24-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 13 of 24-week-old female $Pls3^{fl/fl}$ mice; N = 3 of 48-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 5 of 48-week-old female $Pls3^{fl/fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.

A**12-weeks****B****24-weeks****C****48-weeks**

Supplemental Fig. S10: MicroCT data of spine T12 from male $Pls3^{fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) for 24-, and (C) 48-week-old male mice. N = 12 of 12-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 12-week-old male $Pls3^{fl}$ mice; N = 13 of 24-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 24-week-old male $Pls3^{fl}$ mice; N = 5 of 48-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 4 of 48-week-old male $Pls3^{fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.

A**12-weeks****B****24-weeks****C****48-weeks**

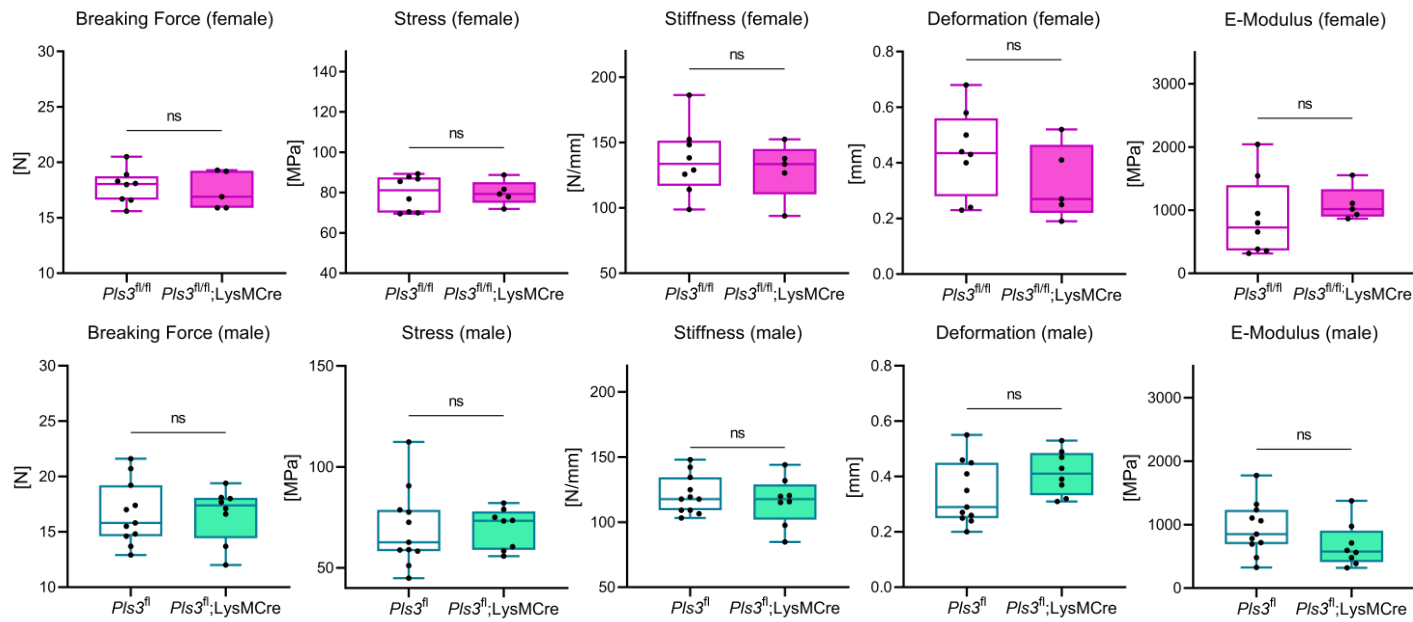
Supplemental Fig. S11: MicroCT data of the spine T13 from female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl/fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) for 24-, and (C) 48-week-old female mice. N = 8 of 12-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 12 of 12-week-old female $Pls3^{fl/fl}$ mice; N = 11 of 24-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 13 of 24-week-old female $Pls3^{fl/fl}$ mice; N = 3 of 48-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 5 of 48-week-old female $Pls3^{fl/fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.

A**12-weeks****B****24-weeks****C****48-weeks**

*Supplemental Fig. S12: MicroCT data of the spine T13 from male $Pls3^{fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl}$ littermates. Shown are bone volume fraction (BV/TV, %), bone mineral density (BMD, mmHA/cm³), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), and connectivity density (Conn.D, 1/mm³), vertebral foramen A/P (mm), vertebral foramen M/L (mm), cross sectional area of the vertebral foramen (mm²) and vertebral body height (mm) for (A) 12-, (B) for 24-, and (C) 48-week-old male mice. N = 12 of 12-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 12-week-old male $Pls3^{fl}$ mice; N = 13 of 24-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 11 of 24-week-old male $Pls3^{fl}$ mice; N = 5 of 48-week-old male $Pls3^{fl};LysMCre^{tg/0}$ mice; N = 4 of 48-week-old male $Pls3^{fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.*

A

48-weeks



Supplemental Fig. S13: 3-point-bending-test (3-PBT) of 48-week-old female and male $Pls3^{fl/fl};LysMCre^{tg/0}$ mice in comparison to their $Pls3^{fl/fl}$ littermates. Shown are breaking force, ultimate stress, stiffness, deformation, and Elastic-modulus (E-modulus). N = 5 of 48-week-old female $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 8 of 48-week-old female $Pls3^{fl/fl}$ mice; N = 8 of 48-week-old male $Pls3^{fl/fl};LysMCre^{tg/0}$ mice; N = 11 of 48-week-old male $Pls3^{fl/fl}$ mice. All results are shown as box plots, representing individual data points with median as a line, interquartile range (25th to 75th percentile), and min to max as whiskers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Statistical test: Mann-Whitney U test.