Supplemental Figure 1. RNF146 deficiency in osteoblasts causes a CCD-like syndrome in mice. (A) Genotyping PCR of Rnf146<sup>+/+</sup>, Rnf146<sup>fl/+</sup>, Rnf146<sup>fl/fl</sup> and Cre recombinase expressing mice using primers shown in the Methods section. The wild-type, floxed (fl) and Cre products are 322 bp, 420 bp and 480 bp, respectively. (B) Representative appearance of Rnf146<sup>fl/fl</sup> and Rnf146<sup>fl/fl</sup> Osx-Cre newborn pups. (C) Calvarium of Rnf146<sup>fl/fl</sup>, Osx-Cre and Rnf146<sup>fl/+</sup> Osx-Cre newborn pups stained by alizarin red and Alcian blue.
Supplemental Figure 2. Osteoblast defect causes osteopenia in Rnf146^{fl/fl} Osx-Cre mice. (A-E) μCT-derived measurements of trabecular thickness (A), cortical major diameter (B), cortical minor diameter (C), cortical periosteal perimeter (D) and cortical endosteal perimeter (E) of 12-week-old Rnf146^{fl/fl} (WT) and Rnf146^{fl/fl} Osx-Cre (KO) mice. n = 5-6. P values were determined by unpaired t-test. Data are presented as mean ± SEM. *P < 0.05.
Supplemental Figure 3. RNF146 is required for osteoblast differentiation. (A) qPCR analysis of Rnf146 mRNA expression in cells in Figure 3C. (B) Whole cell lysates from cells in Figure 3C were probed with the indicated antibodies for Western blot analysis. (C) qPCR analysis of Runx2 mRNA expression in primary murine osteoblasts cultured in serum-free medium in the presence or absence of Wnt3a (40 ng/ml). (D) qPCR analysis of Runx2 mRNA expression in cells in Figure 3C cultured in osteogenic medium for 3-9 days. n = 3. P values were determined by unpaired t-test. Data are presented as mean ± SEM. *P < 0.05.
Supplemental Figure 4. RNF146 regulates osteoblast differentiation through the FGF18-TAZ axis. (A) C2C12 cells were cultured in serum-free medium in the presence or absence of FGF18 (50 ng/ml). Whole cell lysates were probed with the indicated antibodies for Western blot analysis. (B) qPCR analysis of Taz mRNA expression in cells in A. (C) Whole cell lysates from cells in Figure 4D were probed with the indicated antibodies for Western blot analysis. (D) Whole cell lysates from cells in Figure 4H were probed with the indicated antibodies for Western blot analysis. (E) qPCR analysis of Taz mRNA expression in C2C12 cells cultured in serum-free medium in the presence or absence of FGF18 (50 ng/ml) and U0126 (10 µM). (F) Whole cell lysates in cells in E cultured for 10 minutes-24 hours were probed with the indicated antibodies for Western blot analysis. n = 3. P values were determined by ANOVA with Tukey–Kramer’s post-hoc test (E and F) or unpaired t-test (A-C). Data are presented as mean ± SEM. *P < 0.05.
Supplemental Figure 5. RNF146 is required for osteoblast proliferation. (A) Bright-field images of cells in Figure 5A cultured for 2 days. (B) qPCR analysis of Taz mRNA expression in cells in Figure 5B. n = 3. P values were determined by ANOVA with Tukey–Kramer's post-hoc test. Data are presented as mean ± SEM. *P < 0.05. (C) Bright-field images of cells in Figure 5C cultured for 2 days.
Supplemental Figure 6. RNF146 represses adipocyte development and fat stores. (A) Bright-field images of cells in Figure 3C. Black and red arrows indicate osteoblast mineralization and adipocytes, respectively. (B and C) qPCR analysis of Pparg2 (B) or Fabp4 (C) mRNA expression in cells in Figure 3, D-F. n = 3. (D) Culture plate images of Oil-Red-O staining in Figure 6A. (E) Whole cell lysates from cells in Figure 6A were probed with the indicated antibodies for Western blot analysis. (F) Quantification of Western blot analysis in Figure 6B. (G) Culture plate images of Oil-Red-O staining in Figure 6F. (H and I) qPCR analysis of Pparg2 (H) or Fabp4 (I) mRNA expression in cells in Figure 6F cultured in adipogenic medium for 3-9 days. (J) Culture plate images of Oil-Red-O staining in Figure 6G. n = 3. P values were determined by ANOVA with Tukey–Kramer’s post-hoc test (H and I) or unpaired t-test (B, C and F). Data are presented as mean ± SEM. *P < 0.05.
Supplemental Figure 7. *Rnf146*fl/fl Osx-Cre mice have defects in glucose metabolism. (A and B) Histomorphometric analysis of islet number (A) and islet area (B) in pancreas in Figure 7G. Islet number and area represents the number and surface of islet divided by the total pancreas surface, respectively. n = 4-6. P values were determined by unpaired t-test. Data are presented as mean ± SEM. *P < 0.05.