Xiong et al., https://doi.org/10.1083/jcb.201608002

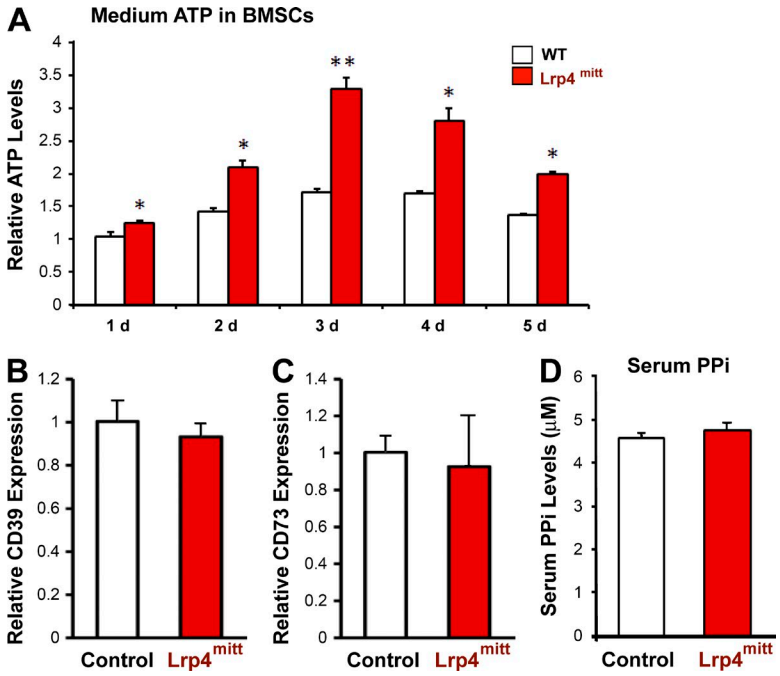

Figure S1. **Increased ATP levels in CMs of Lrp4-deficient BMSCs.** (A) Medium ATP levels in WT and Lrp4-deficient BMSCs were measured using a bioluminescence detection kit from day 1 to 5. Values represent mean ± SD from three different experiments. *, $P < 0.05$; **, $P < 0.001$. (B and C) Real-time PCR analysis of CD39 and CD73 expression in control and $Lrp4^{mitt}$ BMSCs. The values are normalized to β-actin. Mean ± SD values from three different experiments are presented. *, $P < 0.05$. (D) Serum PPi levels in 2-mo-old WT and Lrp4-deficient mice. Shown are mean ± SD values from three different experiments.

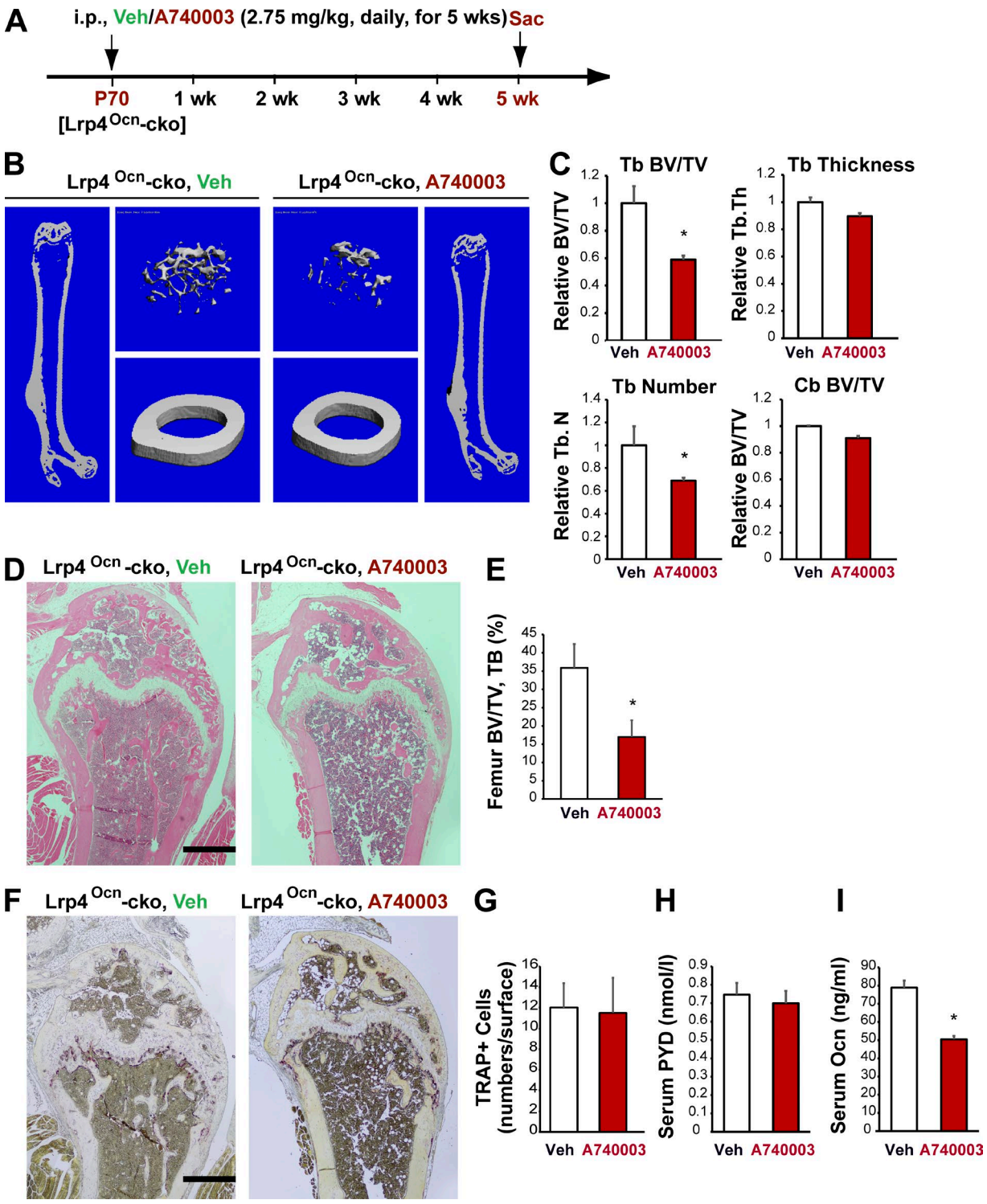

Figure S2. **Decreased trabecular bone mass in Lrp4^{Ocn}-cko mice by A740003, an antagonist of P_2X_7R.** (A) Experimental strategy. Postnatal day 70 (P70) Lrp4^{Ocn}-cko mice were administered a once-daily intraperitoneal injection of 2.75 mg/kg A740003 or vehicle (0.9% sodium chloride) for 5 wk. The femurs and sera samples collected after treatments were subjected to µCT and ELISA/RIA assays, respectively. (B and C) The µCT analysis of femurs from Lrp4^{Ocn}-cko littermates. Five different male mice of each genotype per group were examined blindly. Representative 3D images are shown in B. Quantification analyses of trabecular bone (TB) volumes over total volumes (BV/TV), TB thickness (TB. Th), TB number (Tb. N), and cortical bone BV/TV by direct model of µCT analysis are presented in C. Data were analyzed by two-way ANOVA; *, $P < 0.05$. (D and E) Histomorphological examinations of femur bone mass by hematoxylin and eosin (H&E) staining analysis. Bar, 500 µm. Quantification analyses of femur trabecular BV/TV ($n = 5$ bone samples for each group) are presented in E. Mean ± SD values ($n = 5$ animals per genotype) are presented. *, $P < 0.05$. (F and G) TRAP staining analysis of femur sections. Bar, 500 µm. Quantification analysis is shown in G as mean ± SD ($n = 5$ femur samples for each group). (H and I) RIA analysis of serum pyridinoline (PYD) and osteocalcin (Ocn) levels. Values represent mean ± SD ($n = 5$). *, $P < 0.05$.

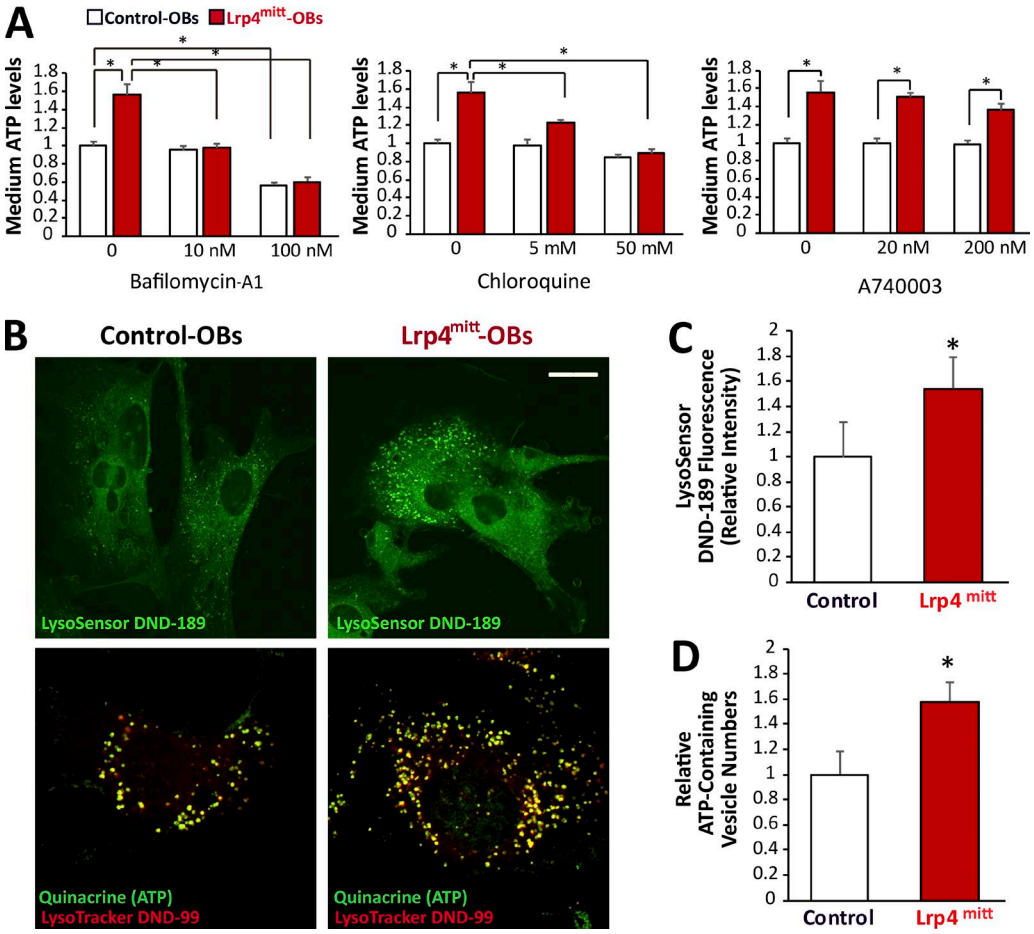

Figure S3. **Elevated v-ATPase–driven vesicular ATP loading and release in Lrp4-deficient OBs.** (A) Primary cultured control and Lrp4-deficient OBs were treated with sham (PBS), BafA1, chloroquine, or A740003 for the indicated dose. After 2-h treatment, ATP levels in the culture medium were measured using a bioluminescence detection kit. Mean ± SD values from three different experiments are shown. *, $P < 0.05$. (B–D) Increased vesicular acidification and ATP loading in Lrp4-deficient OBs. (B) ATP-containing vesicles were labeled with quinacrine (green channel), and lysosomes were stained with LysoTracker DND-99 (red channel). Bar, 10 µm. The green fluorescence intensity of LysoSensor was measured using ImageJ (mean ± SD; $n = 20$) in C. *, $P < 0.05$. Quantification analysis of ATP-containing vesicle numbers is shown in D. Values represent mean ± SD, $n = 20$; *, $P < 0.05$.

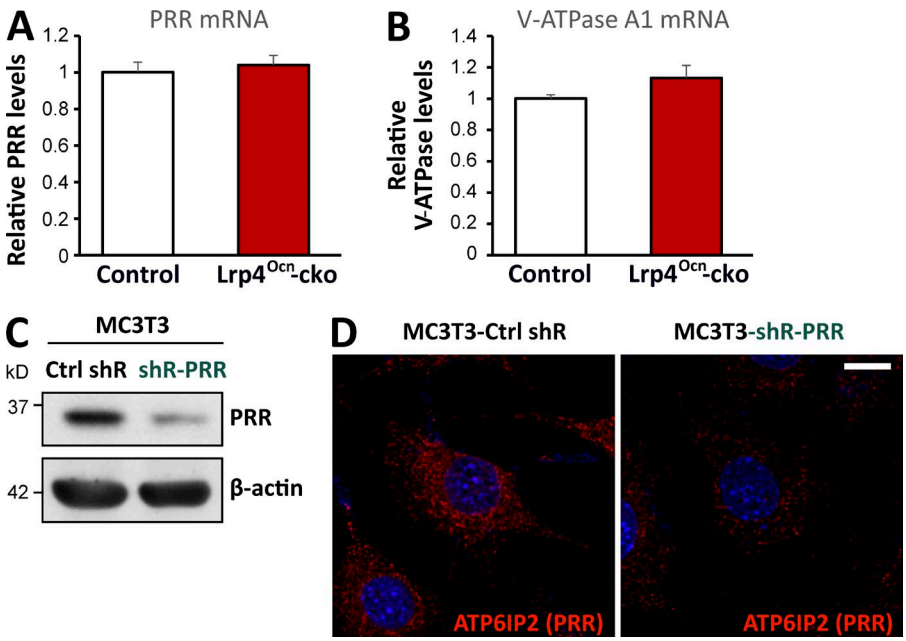

Figure S4. **PRR expression in BMSCs or PRR knockdown in MC3T3 cells.** (A and B) Real-time PCR analysis of PRR and V-ATPase A1 expression in Lrp4^{Ocn}-cko BMSCs. The values are normalized to β-actin. Mean ± SD values from three different experiments are presented. (C) MC3T3-E1 cells were infected with lentiviruses expressing scramble control or shRNA-PRR and purified. Cell lysates were subjected to Western blot analysis. (D) MC3T3-Ctrl-shR and MC3T3-shR-PRR were fixed and subjected to immunostaining analysis using anti-PRR antibody. Bar, 10 µm.

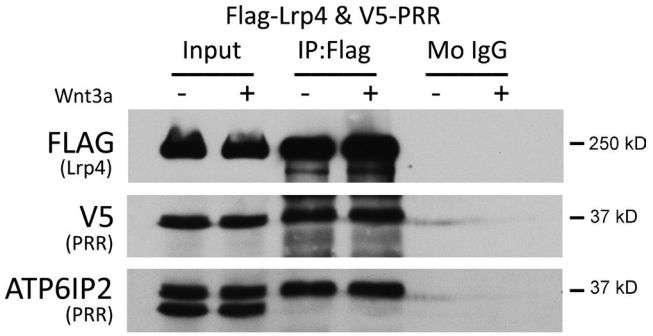

Figure S5. **Lrp4 interaction with PRR.** HEK293T cells were transfected with Flag-Lrp4 and V5-PRR. 48 h after transfection, cells were treated with PBS or Wnt3a for 1 h. Cell lysates (~500 µg) were immunoprecipitated by anti-Flag antibody (IP: Flag group) or mouse IgG (Mo IgG group). The resulting lysates were subjected to Western blot analysis using indicated antibodies. ~50 µg of cell lysates was used as an input. The data presented are representative of three independent experiments.