Schmidt et al., http://www.jcb.org/cgi/content/full/jcb.201007141/DC1

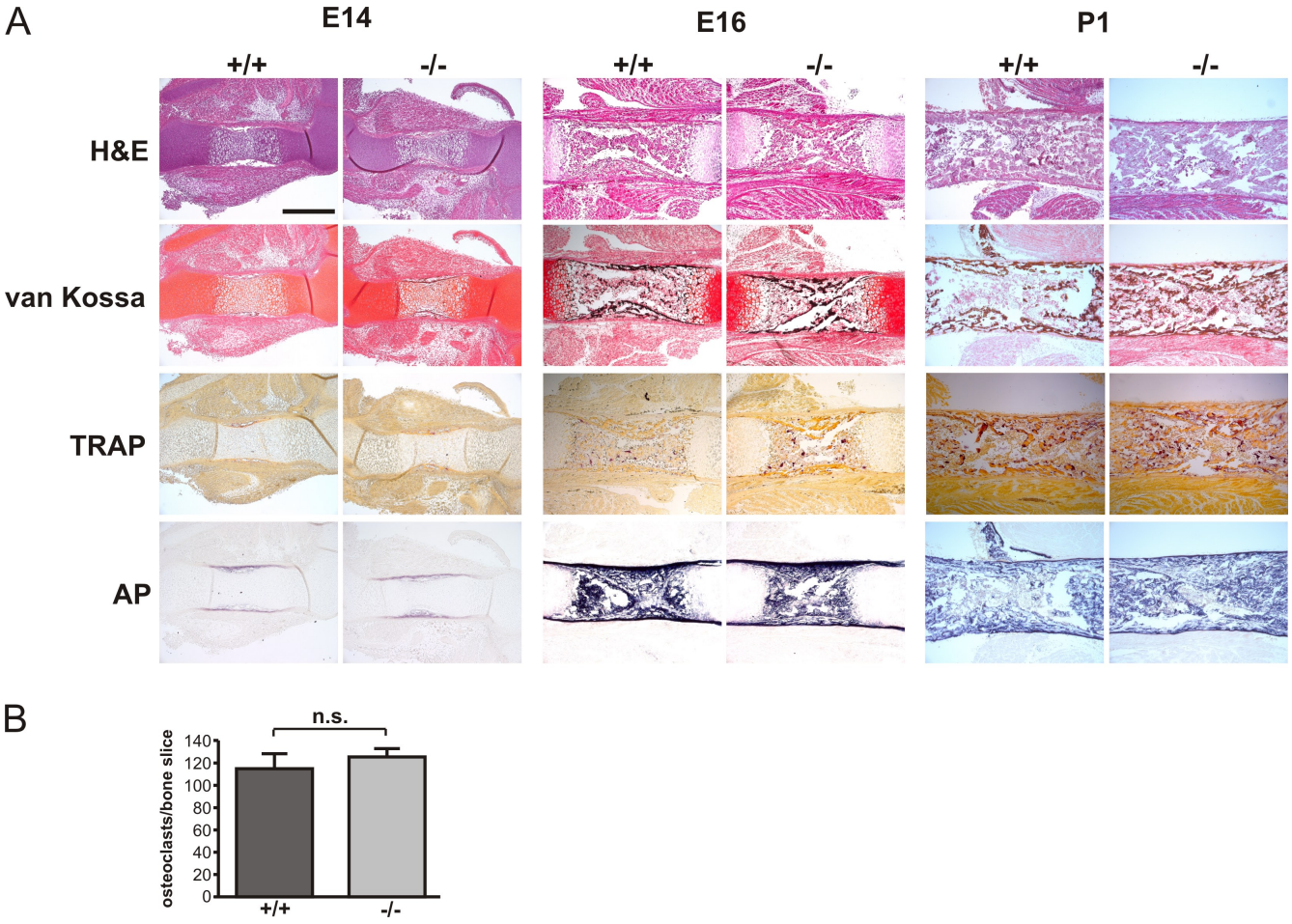

Figure S1. **Fetal development of osteopetrosis.** (A) Histology of femora from wild-type and kindlin-3$^{-/-}$ embryos at E14, E16, and P1. Consecutive sections stained with hematoxylin and eosin (H&E), van Kossa, TRAP, and AP activity. Bar, 200 µm. (B) Number of TRAP-positive osteoclasts in histological sections from wild-type and kindlin-3$^{-/-}$ femora at P1. n.s., not significant.

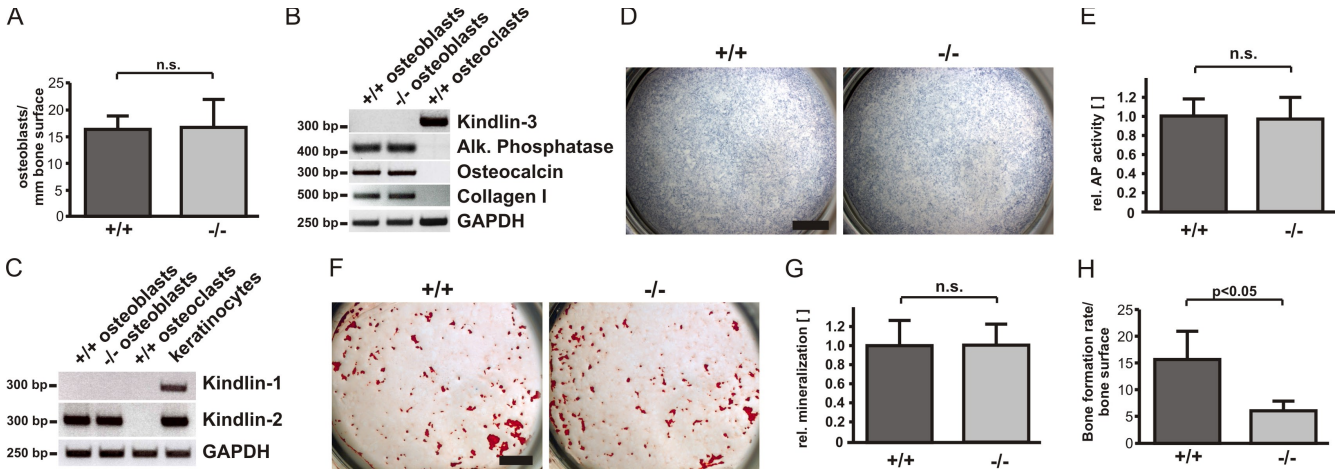

Figure S2. **Osteoblasts are normal in kindlin-3$^{-/-}$ mice.** (A) Histomorphometric measurement of osteoblast number per bone surface in tibiae of P2 wild-type and kindlin-3$^{-/-}$ mice; *n* = 4. (B) RT-PCR of osteoblast markers and kindlin-3 in primary wild-type and kindlin-3$^{-/-}$ osteoblasts and wild-type osteoclasts. (C) RT-PCR of kindlin-1 and -2 in wild-type and kindlin-3$^{-/-}$ osteoblasts and control osteoclasts. RNA from keratinocytes was used as a control. (D) Primary calvarial osteoblasts from wild-type and kindlin-3$^{-/-}$ newborn mice stained for AP. Bar, 3 mm. (E) Relative AP activity in lysates from primary wild-type and kindlin-3$^{-/-}$ osteoblasts was measured photometrically at 405 nm; *n* = 10/4. (F) Bone nodule formation by cultured wild-type and kindlin-3$^{-/-}$ osteoblasts visualized by Alizarin red S staining. Bar, 3 mm. (G) Quantification of mineralization after Alizarin red S dye extraction and photometric measurement at 405 nm; *n* = 6/4. (H) Bone formation rate corrected to bone surface measured by histomorphometry; *n* = 4. Data are presented as mean ± SD (error bars).

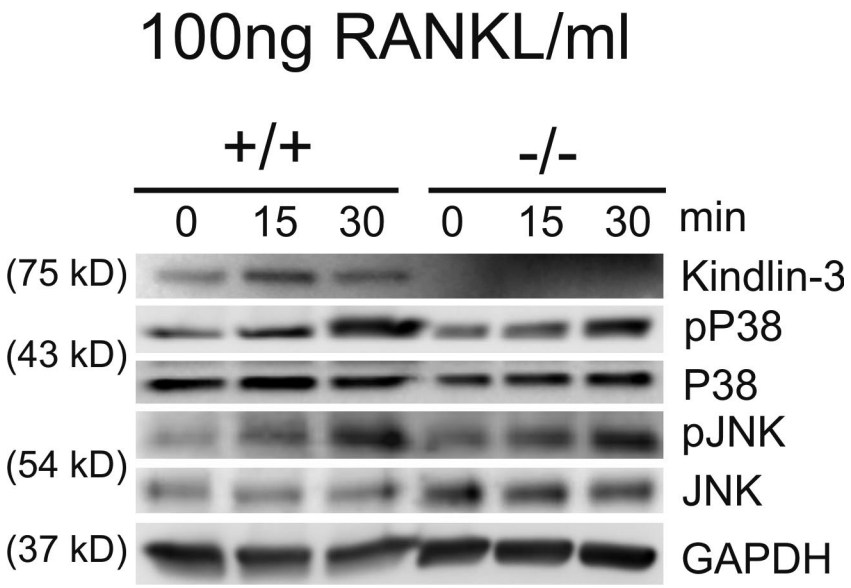

Figure S3. **Normal RANKL signaling in kindlin-3$^{-/-}$ osteoclasts.** Starved wild-type and kindlin-3$^{-/-}$ osteoclasts treated with 100 ng/ml RANKL for the indicated time periods. Western blot analyses for p-p38 and p-JNK are shown.

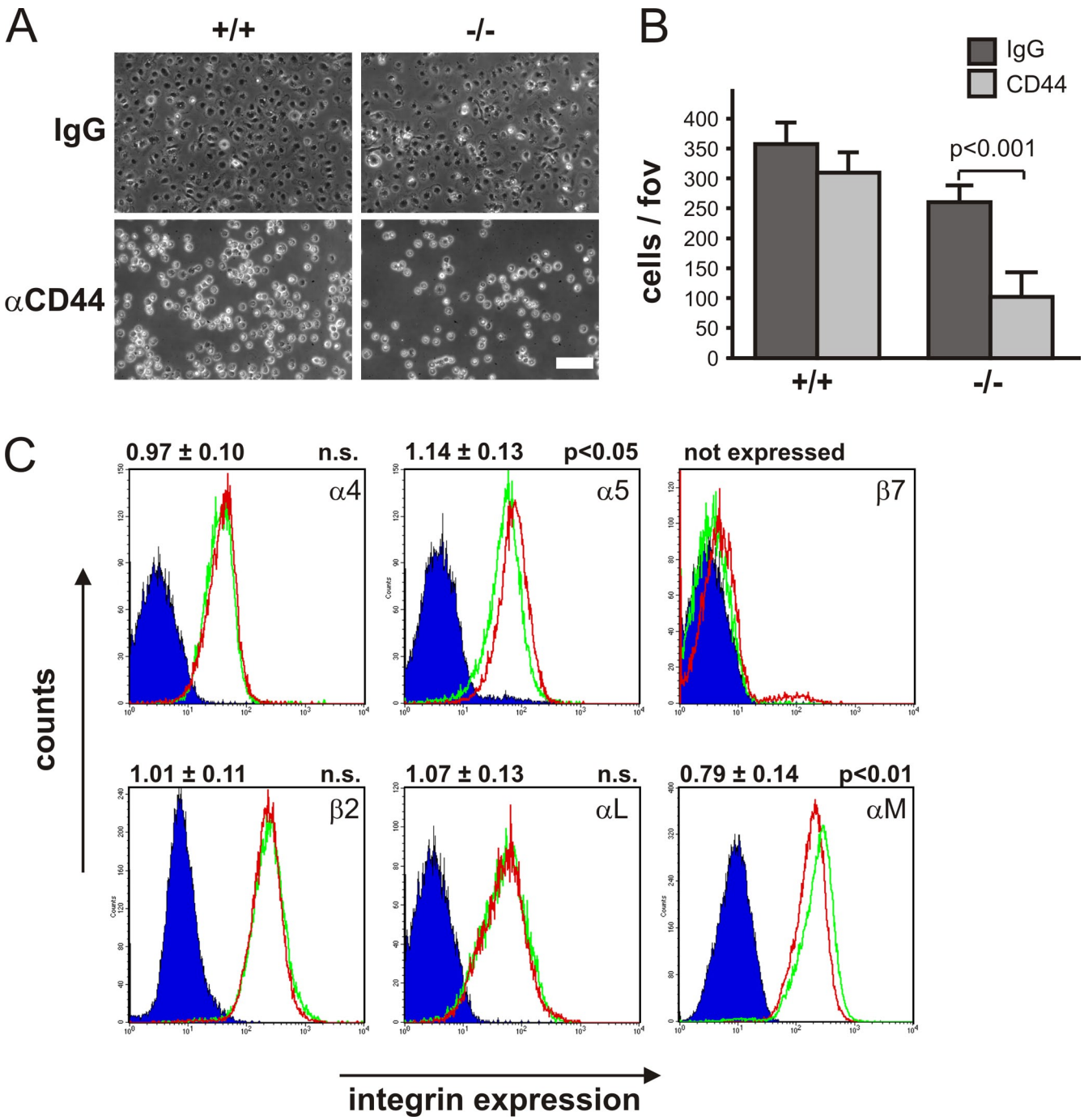

Figure S4. **CD44-mediated adhesion of kindlin-3$^{-/-}$ pre-osteoclasts and integrin expression levels on kindlin-3$^{-/-}$ macrophages.** (A and B) Wild-type and kindlin-3$^{-/-}$ pre-osteoclasts were treated with either an isotope control or an α-CD44 antibody and plated on fibronectin. Cells were imaged (A) and quantified (B) after gentle washing and fixation; $n = 4$. Bar, 100 µm. (C) α4, α5, αL, αM, β2, and β7 integrin surface expression of wild-type (green) and kindlin-3$^{-/-}$ (red) macrophages. Isotype control staining is shown in dark blue. Data are presented as mean ± SD (error bars). P-values indicate significant differences from wild-type (Student's *t* test).

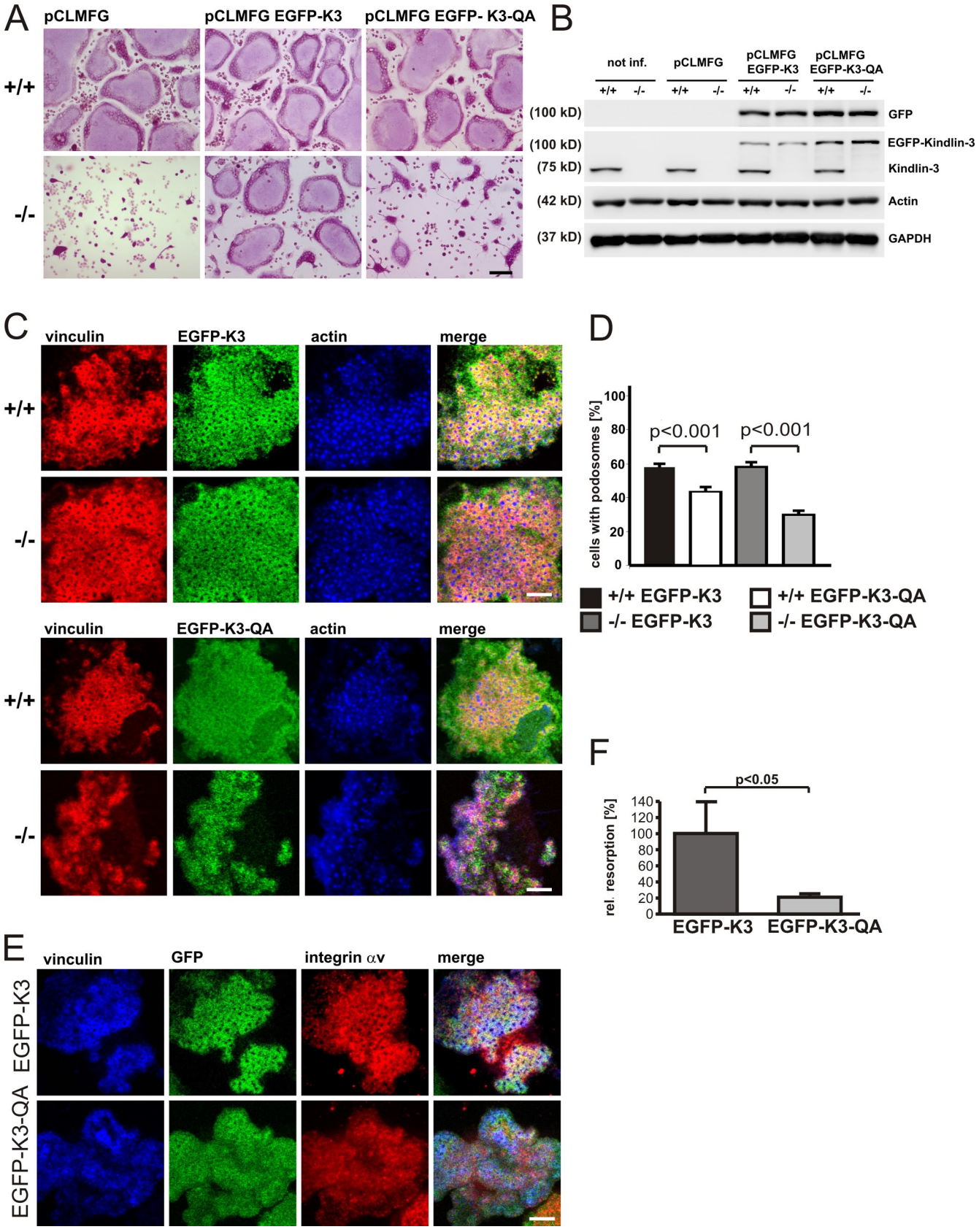

Figure S5. **Re-expression of wild-type and an integrin-binding mutant kindlin-3 in kindlin-3$^{-/-}$ osteoclasts.** (A) TRAP staining of wild-type and kindlin-3$^{-/-}$ osteoclasts transduced with either control virus (pCLMFG), or viruses expressing EGFP–kindlin-3 or an integrin-binding mutant EGFP–kindlin-3–QA. Bar, 100 μm. (B) Western blotting for GFP and kindlin-3 indicates relative expression levels of endogenous and retroviral-induced wild-type and mutant kindlin-3 expression in osteoclasts. GAPDH and actin served as loading controls. (C) Vinculin (red) and F-actin staining (phalloidin, blue) of wild-type and kindlin-3$^{-/-}$ osteoclasts transduced with an EGFP–kindlin-3 or an EGFP–kindlin-3 binding mutant EGFP–kindlin-3–QA (green). Bar, 5 μm. (D) The percentage of infected fetal liver cells that formed podosomes was quantified in four independent experiments. (E) Vinculin (blue) and integrin αv (red) staining of kindlin-3$^{-/-}$ osteoclasts transduced with viruses expressing EGFP–kindlin-3 or mutant EGFP–kindlin-3–QA (green). Bar, 5 μm. (F) Resorption activity of kindlin-3$^{-/-}$ osteoclasts transduced with an EGFP–kindlin-3 or mutant EGFP–kindlin-3–QA–expressing virus on calcium apatite-coated slides quantified with MetaMorph; $n = 3$. Data are presented as mean ± SD (error bars). P-values indicate significant differences from wild-type (Student's *t* test).