SUPPLEMENTARY FIGURES

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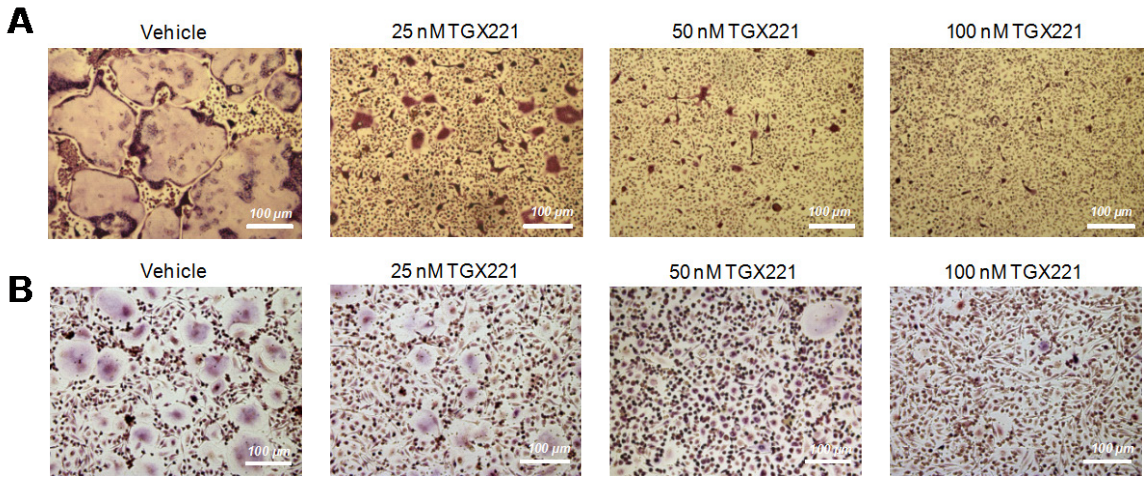
Figure S1



Pharmacological inhibition of PI3Kβ blocks development of murine and human osteoclasts
A-B) Representative images of TRAP-stained wild type mouse bone marrow-derived (A) or human blood mononuclear cell-derived (B) osteoclasts cultured for 3 (A) or 14 (B) days in the presence of 50 ng/ml M-CSF, 50 ng/ml RANKL and the indicated concentrations of TGX221 or 0.1% ethanol (Vehicle). Osteoclasts were defined as TRAP-positive cells with 3 or more nuclei. Images are representative of 20 (A) or 4 (B) independent experiments. Quantification of the results are shown in Figure 1.

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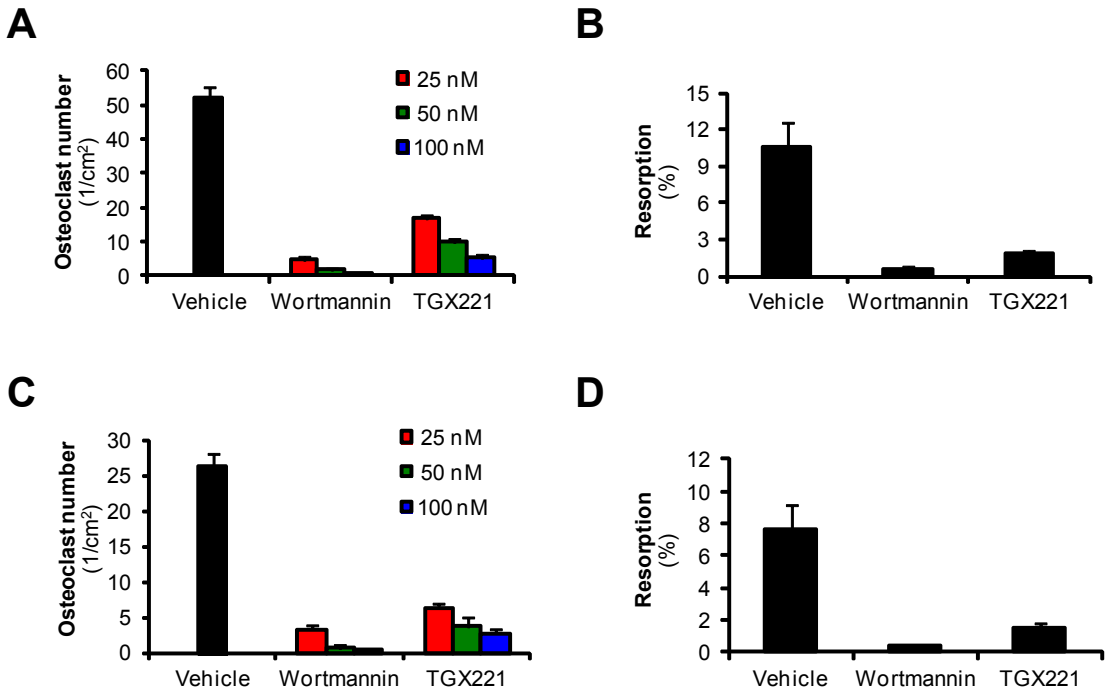
Figure S2



Effect of PI3Kβ inhibition on osteoclast development and function at low cytokine levels
Quantification of TRAP-stained cell cultures (A,C) of, and *in vitro* resorption pit formation (B,D) by, wild type mouse bone marrow-derived (A-B) or human blood mononuclear cell-derived (C-D) osteoclasts cultured for 3 (A), 11 (B) or 14 (C-D) days in the presence of 20 ng/ml M-CSF, 20 ng/ml RANKL and the indicated concentration (or, in (B) and (D), 50 nM) PI3-kinase inhibitors or 0.1% vehicle. Resorption pits appear as lighter areas. Osteoclasts were defined as TRAP-positive cells with 3 or more nuclei. *In vitro* resorption was defined as the percentage of resorbed area. Bar graphs show mean and SEM of data from 3 independent experiments.

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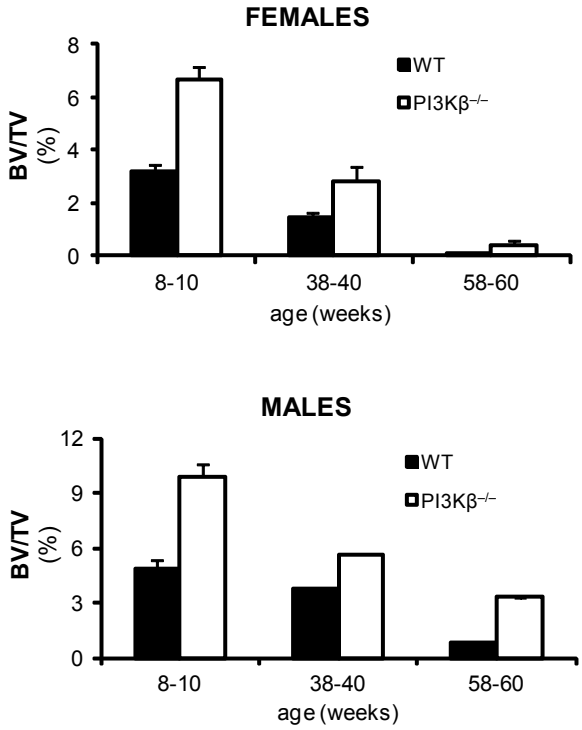
Figure S3



PI3Kβ is required for in vivo bone homeostasis in mice of different age groups
Quantitative micro-CT analysis of the trabecular bone architecture of wild tpe (WT) and PI3Kβ$^{-/-}$ female and male mice of the indicated ages. Data were obtained from a total of 26 mice per genotype. Error bars represent SEM. BV/TV, percent bone volume (bone volume/total volume).

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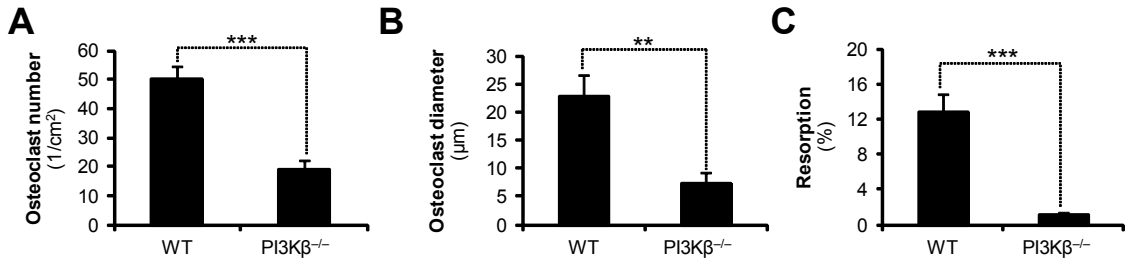
Figure S4



Effect of PI3Kβ deficiency on osteoclast development and function at low cytokine levels
A-C) Quantification of TRAP-stained cell cultures (A-B) of, and *in vitro* resorption pit formation on artificial hydroxyapatite (C) by, wild type (WT) and PI3Kβ$^{-/-}$ mouse bone marrow-derived osteoclasts cultured for 3 (A-B) or 11 (C) days in the presence of 20 ng/ml M-CSF and 20 ng/ml RANKL. Bar graphs represent mean and SEM of data from 3 independent experiments. **, $p < 0.01$; ***, $p < 0.002$.

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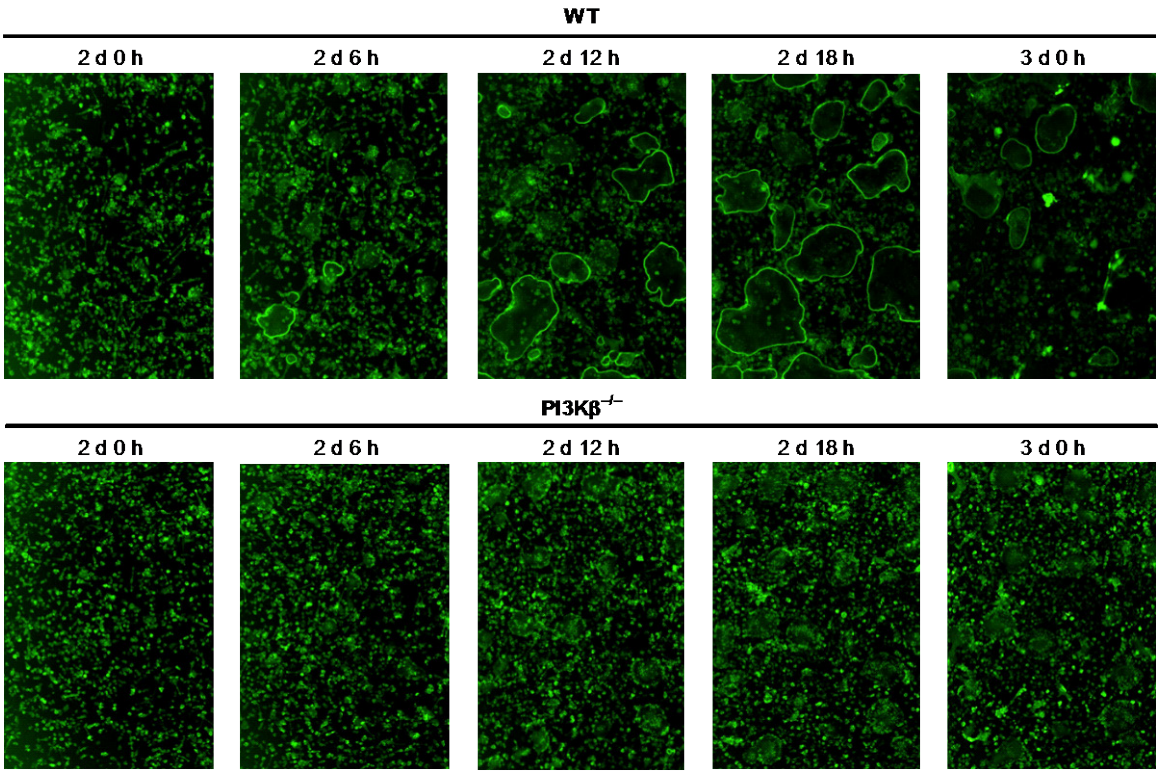
Figure S5



Snapshots of F-actin dynamics in wild type and PI3Kβ$^{-/-}$ osteoclast cultures
Snapshots of Supplementary Videos 1 and 2 showing Lifeact-EGFP fluorescence of wild type (WT; Video 1) and PI3Kβ$^{-/-}$ (Video 2) mouse bone marrow-derived osteoclasts cultures, taken at the indicated time points after addition of RANKL. See description of the videos for further details.

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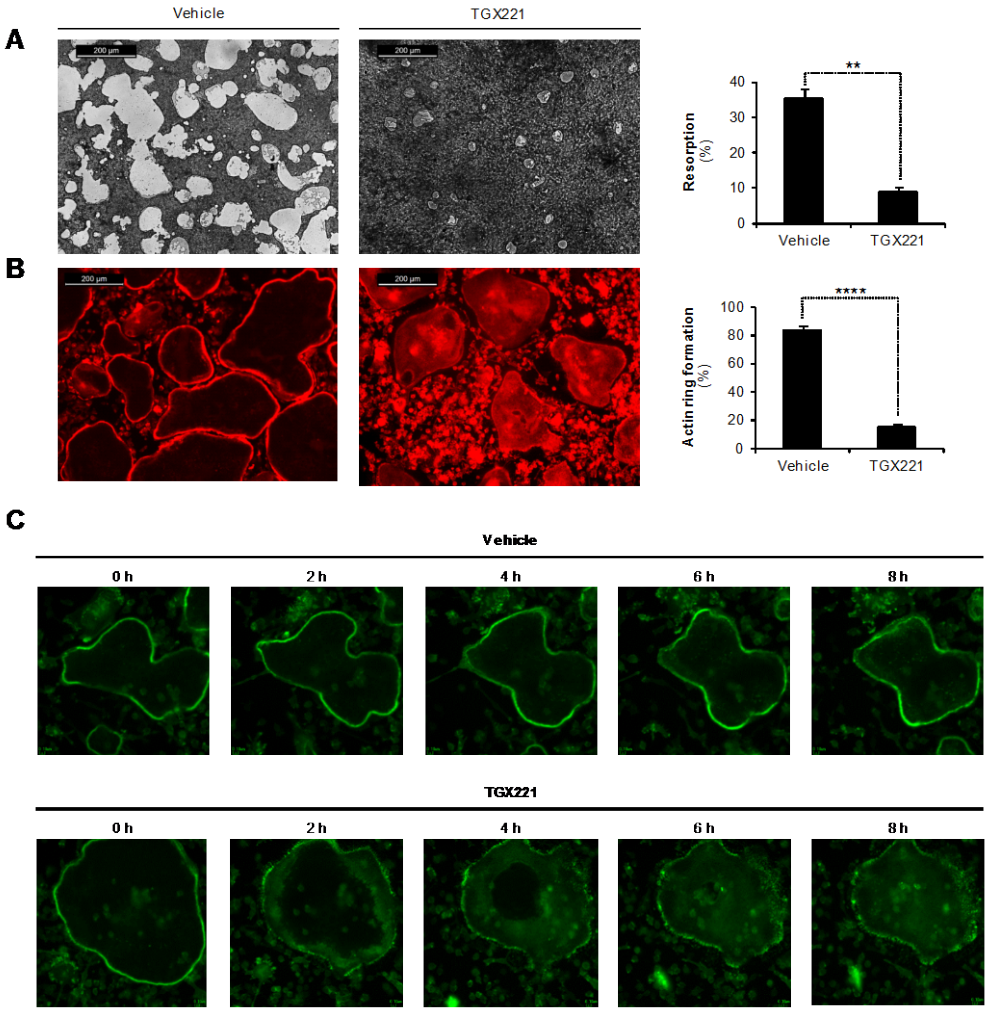
Figure S6



Pharmacological inhibition of PI3Kβ after osteoclast formation blocks resorptive activity and actin ring maintenance

A) Representative images and quantification of *in vitro* resorption pit formation by wild type mouse bone marrow-derived osteoclasts cultured on an artificial hydroxyapatite layer for 8 days in the presence of 50 ng/ml M-CSF and 50 ng/ml RANKL, with 50 nM TGX221 or 0.1% ethanol (vehicle) present during the last 5 days. Resorption pits appear as lighter areas. **B)** Representative fluorescence images and quantification of wild type mouse bone marrow-derived osteoclasts differentiated in the presence of 50 ng/ml M-CSF and 50 ng/ml RANKL for 3 days, treated with 50 nM TGX221 or 0.1% ethanol (vehicle) in the continued presence of cytokines for 6 additional hours, and then stained with rhodamine-conjugated phalloidin. Images in A-B are representative of 3 independent experiments. Quantitative data show mean and SEM from 3 independent experiments. **, $p < 0.01$; ****, $p < 0.0004$. **C)** Snapshots of Supplementary Videos 3 and 4 showing fluorescence of 0.1% ethanol (vehicle; Video 3) and TGX221 (Video 4) treated Lifeact-EGFP expressing mouse bone marrow-derived osteoclasts cultures. See description of the videos for further details.

SUPPLEMENTARY VIDEOS

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Supplementary Video 1 and 2
F-actin dynamics in wild-type (Video 1) and PI3Kβ$^{-/-}$ (Video 2) osteoclast cultures
Visualization of actin ring formation by Lifeact-EGFP expressing wild type (WT) and PI3Kβ$^{-/-}$ mouse bone marrow-derived osteoclasts cultured in the presence of 50 ng/ml M-CSF and 50 ng/ml RANKL. Lifeact-EGFP: green. The videos are representative of 3 independent experiments. Time-lapse imaging was performed using an Essen BioScience IncuCyte Zoom imaging system. Supplementary videos 1 and 2 are available on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38660/suppinfo.

Supplementary Video 3 and 4
F-actin dynamics in wild-type osteoclast cultures treated with vehicle (Video 3) and TGX221 (Video 4)
Visualization of actin ring formation by Lifeact-EGFP expressing wild type mouse bone marrow-derived osteoclasts differentiated in the presence of 50 ng/ml M-CSF and 50 ng/ml RANKL for 3 days, then treated with 0.1% ethanol (vehicle) or 50 nM TGX221 in the continued presence of cytokines for an additional 8 hours. Lifeact-EGFP: green. The videos are representative of 2 independent experiments. Time-lapse imaging was performed using a Nikon BioStation IM-Q imaging system. Supplementary videos 1 and 2 are available on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38660/suppinfo.