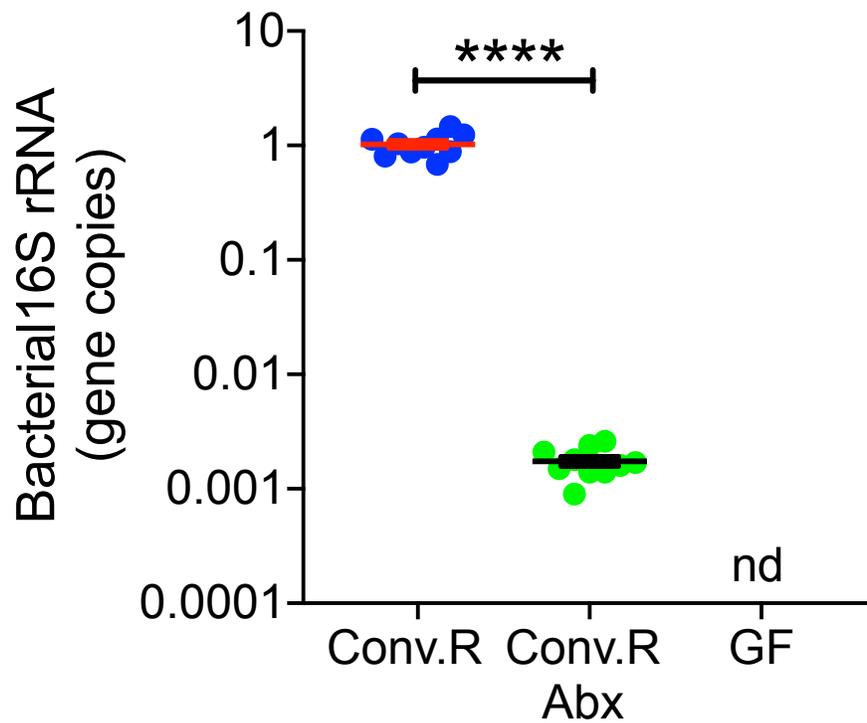
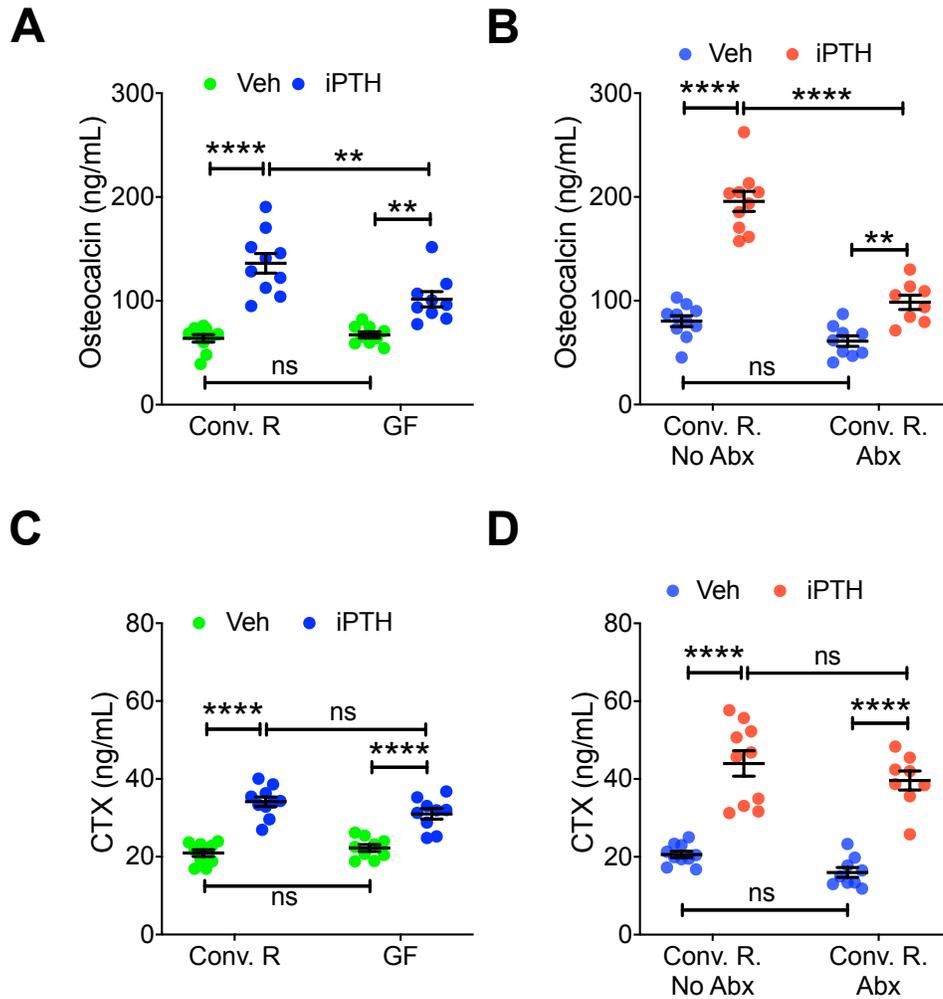


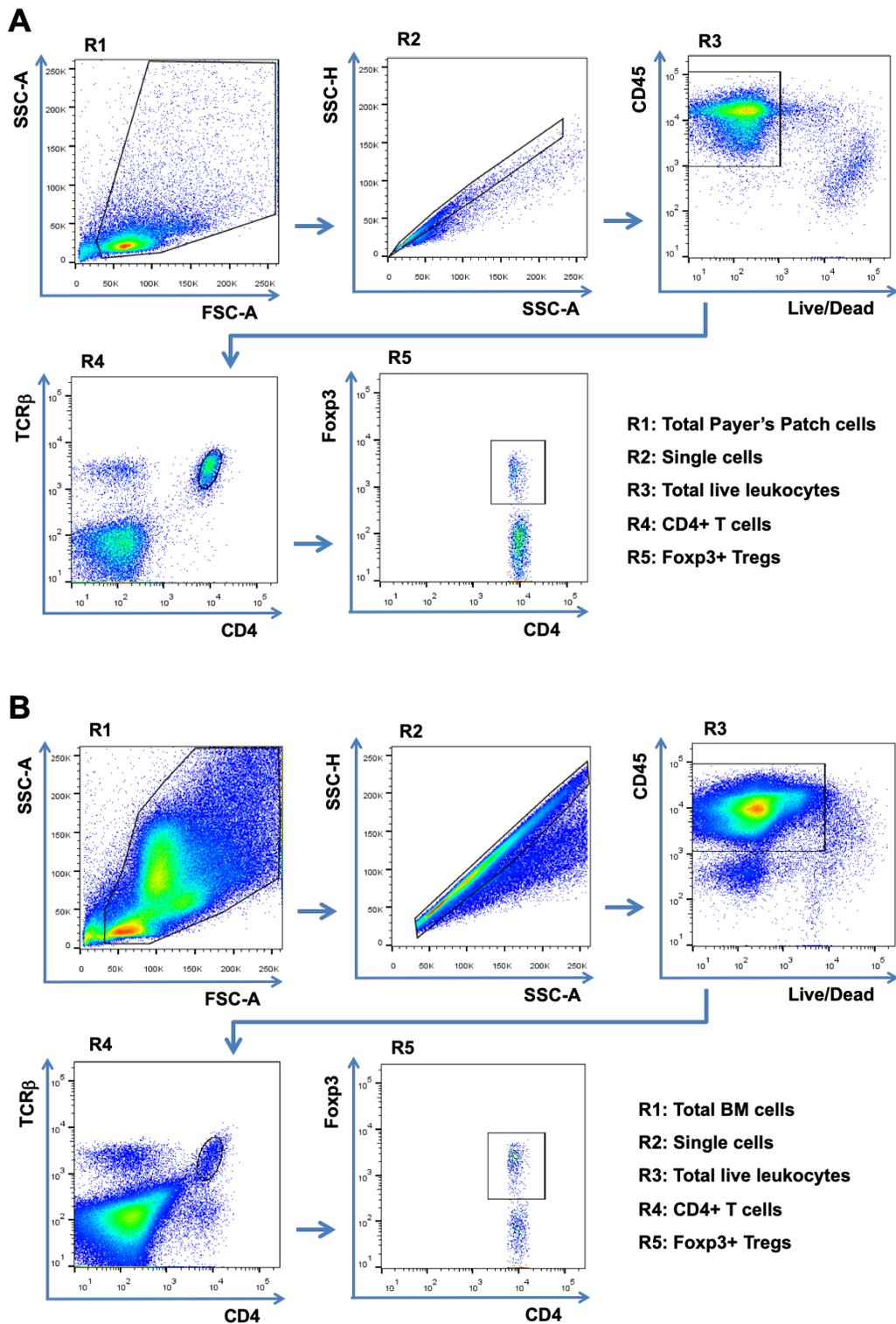
Supplemental figure 1. iPTH does not improve indices of trabecular bone structure in young and old Conv. R. mice treated with antibiotics (Abx) and in GF mice. The figure shows measurements of femoral indices of trabecular structure by in vitro μ CT scanning. **A,B.** Trabecular Number (Tb.N) and Trabecular Separation (Tb.Sp) in 12-week-old Conv. R. mice and GF mice. **C,D.** Tb.N and Tb.Sp in 12-week-old Conv. R. mice treated or not treated with Abx. **E,F.** Tb.N and Tb.Sp in 7-month-old Conv. R. mice treated or not treated with Abx. $n = 8-10$ mice per group. Data are expressed as Mean \pm SEM. All data were normally distributed according to the Shapiro-Wilk normality test. Data were analyzed by two-way ANOVA and post hoc tests applying the Bonferroni correction for multiple comparisons. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ and **** = $p < 0.0001$ compared to the indicated group. ns = not significant.



Supplemental figure 2. Number of universal bacteria in Conv. R mice treated with antibiotics (Abx) and in GF mice. Genomic DNA was isolated from equal amounts of fecal material of Conv. R mice, Conv. R mice treated with Abx, and GF mice. Relative quantitation of 16S rRNA gene copies of total bacteria were determined by qPCR, using universal 16S rRNA primers. n = 10 mice per group. Data are expressed as Mean \pm SEM. Data were analyzed by unpaired t-tests. **** = p < 0.0001. nd = not detectable. nd = not detectable.



Supplemental figure 3. Microbiota depletion by antibiotic treatment does not block the capacity of iPTH to increases biochemical markers of bone turnover. **A,B.** Serum levels of osteocalcin (OCN), a marker of bone formation. **C,D.** Serum levels of type 1 cross-linked C-telopeptide (CTX), a marker of resorption. GF mice and Conv. R. mice were treated with iPTH or vehicle for 4 weeks. Conv. R. mice were also treated with and without antibiotics (Abx). Mice were sacrificed and analyzed at 12 weeks of age. n = 8-10 mice per group. Data are expressed as Mean \pm SEM. All data were normally distributed according to the Shapiro-Wilk normality test. Data were analyzed by two-way ANOVA and post hoc tests applying the Bonferroni correction for multiple comparisons. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ and **** = $p < 0.0001$ compared to the indicated group. ns = not significant.



Supplemental figure 4. Gating strategy used to identify PP (Panel A) and BM. (Panel B) Treg cells by flow cytometry. Following red cells lysis, single cell suspensions were prepared from BM, and stained with antibodies to the indicated antigens and live/dead cell dye. Gating regions are numbered from R1 to R5. The figure shows one representative gating of flow cytometric plot.

