Supplemental Figure 1. Effects of Leuprolide and treatment with probiotics on uterus weight and body weight. Data are expressed as Mean ± SEM. All data were normally distributed according to the Shapiro-Wilk normality test and analyzed by two-way analysis-of-variance and post hoc tests applying the Bonferroni correction for multiple comparisons.

A. Effects of Leuprolide on the uterus weight in Conv. R, GF and Col. Mice. n = 10 mice per group. **** = p<0.0001 compared to the corresponding vehicle group.

B. Effects of probiotic supplementation on body weight in sham operated and ovx mice. n=10-14 mice per group. **** = p<0.0001 compared to sham vehicle group.

C. Effects of probiotic supplementation on uterus weight in sham operated and ovx mice. n=10-14 mice per group **** = p<0.0001 compared to sham vehicle group. In panels B and C conventional ovariectomized (ovx) and sham operated mice were supplemented twice a week with 1x10^9 cfu of either VSL#3™, Lactobacillus rhamnosus GG (LGG), an LGG pili mutant LGG(Δ SpaC) referred to as LGG-M, E. coli, or vehicle.
Supplemental Figure 2. Effects of Leuprolide treatment on the generation of osteoclastogenic cytokines by BM T cells. A-B. Levels of IFNγ and IL-4 in the bone marrow (BM) of conventionally raised (Conv.R), of germ-free (GF), and of colonized GF (Col.GF) mice following either Leuprolide (375 µg/month) or vehicle control treatment for 10 weeks. C-D. RT-qPCR analysis measuring transcript levels of IFNγ and IL-4 in the small intestine of mice in experimental groups described in A-B. n = 10 mice per group. Data were normally distributed according to the Shapiro-Wilk normality test and analyzed by two-way analysis-of-variance and post hoc tests applying the Bonferroni correction for multiple comparisons. * = p <0.05, and **= p<0.01, compared to the indicated group.
Supplemental Figure 3. Effects of Leuprolide treatment on the generation of osteoclastogenic cytokines by BM T cells. RT-qPCR analysis measuring transcript levels of TNF, RANKL and IL-17 in purified BM T cells. Data are expressed as Mean ± SEM. n = 10 mice per group. Data were normally distributed according to the Shapiro-Wilk normality test and analyzed by two-way analysis-of-variance and post hoc tests applying the Bonferroni correction for multiple comparisons. * = p < 0.05, **= p<0.01, ***= p<0.001 and ****= compared to the indicated group.
Supplemental Figure 4. Supplementation of the indigenous microbiota with probiotics prevents the alterations of bone structure induced by sex-steroid deficiency. Conventional ovariectomized (ovx) and sham operated mice were supplemented twice a week with 1x10^8 cfu of either VSL#3™, Lactobacillus rehamnosus GG (LGG), an LGG pili mutant LGG(ΔSpaC) referred to as LGG-M, E. coli, or vehicle. Data are expressed as Mean ± SEM. All data were normally distributed according to the Shapiro-Wilk normality test and analyzed by ANOVA for repeated measures and post hoc tests applying the Bonferroni correction for multiple comparisons. n = 10 to 14 mice per group. **A-C.** In vivo prospective analysis of spinal trabecular thickness (Tb.Th), trabecular space (Tb.Sp), and trabecular number (Tb.N) by µCT scanning at baseline and 2 and 4 weeks after surgery. a = p < 0.05, aa= p<0.01 aaa= p<0.001 and aaaa= p<0.0001 compared to baseline. * = p < 0.05, **= p<0.01 ***= p<0.001 and ****= p<0.0001 compared to sham vehicle, # = p < 0.05, ##= p<0.01 ###= p<0.001 and ####= p<0.0001 compared to ovx vehicle. **D-G.** In vitro analysis of femoral indices of trabecular structure (Tb.Th, Tb.N, and Tb.Sp) and cortical structure (Ct.Th) by µCT scanning. * = p < 0.05, **= p<0.01, ***= p<0.001 and ****= p<0.0001 compared to sham vehicle, # = p < 0.05,##= p<0.01 , ###= p<0.001 and ####= p<0.0001 compared to ovx vehicle.
Supplemental Figure 5. Supplementation of the indigenous microbiota with probiotics modulates the generation of osteoclastogenic cytokines by BM T cells following sex-steroid depletion. RT-qPCR analysis measuring transcript levels of TNF, RANKL and IL-17 in purified BM T cells. n = 10 mice per group in all panels. Data are expressed as Mean ± SEM. All data were normally distributed according to the Shapiro-Wilk normality test and analyzed by two-way analysis-of-variance and post hoc tests applying the Bonferroni correction for multiple comparisons. ***= p<0.001 compared to sham vehicle, ##= p<0.01 compared to ovx vehicle.