Supplementary information, Figure S2

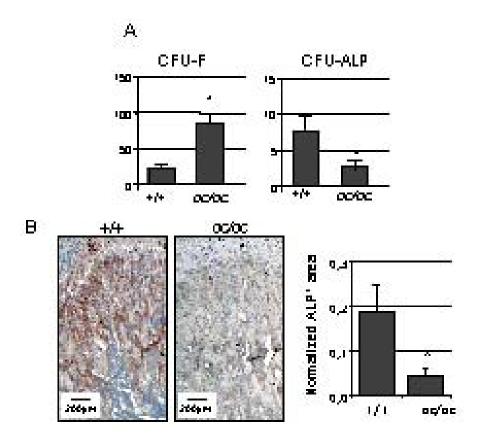


Figure S2 Reduced alkaline phosphatase expression in the femora of oc/oc mice. (A) Total bone marrow cells from the femora of oc/oc and +/+ mice were seeded in 12 well plates at 5×10^5 cells/well in α MEM supplemented with penicillin, streptomycin and 10% fetal bovine serum (Hyclone) for 20 days. For CFU-F enumeration, cells were stained 30 sec with hematoxillin. For ALP enumeration cells were stained for ALP activity according to the manufacturer protocol (leukocyte alkaline phosphatase kit, Sigma) for 30 min. Colonies were enumerated using a light microscope (Axiovert, Zeiss). Results are representative of the mean ± SD of triplicates from 3 mice in each group. (*p < 0.01). (B) Femora were fixed in 4% paraformaldehyde for 24h at 4 °C, decalcified in 10% EDTA for 10 days at 4 °C and incubated overnight in a 30% sucrose solution and 8µm sections were obtained with a cryotome (ThermoShandon). Bone sections were stained for alkaline phosphatase activity with the leukocyte alkaline phosphatase kit (Sigma). Image acquisition was performed on a Zeiss Axio Observer Z1 microscope (Visitron Systems GmbH), with the AxioVision software (Carl Zeiss) in the same conditions.