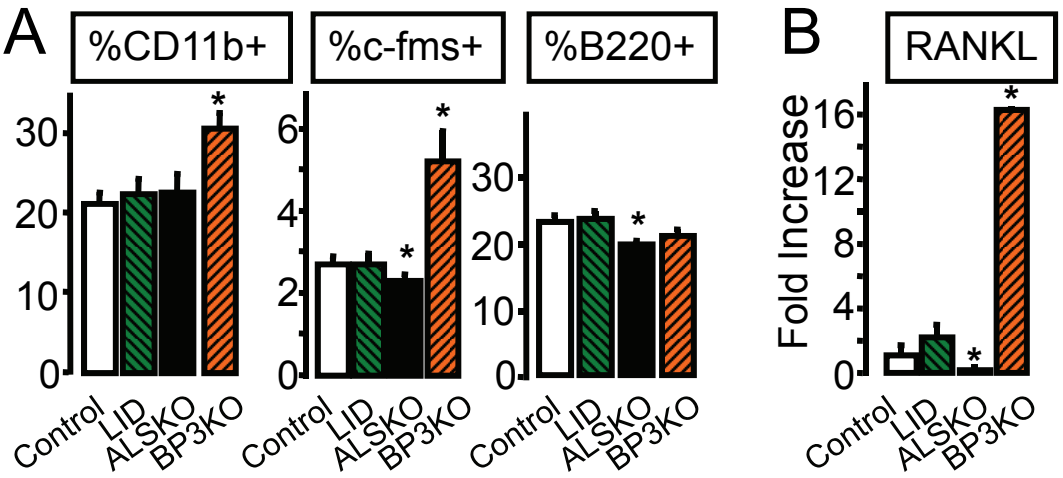

Supp. Fig. 2. FACS analysis of bone marrow (BM) reveal an enhanced potential for osteoclastogenesis in BP3KO mice, but attenuation of this process in ALSKO mice. (A) A significantly greater fraction of cells expressing CD11b+ and mouse colony-stimulating factor-1 (M-CSF1) receptor (i.e., c-fms), which is a critical factor for osteoclastogenesis, is found in marrow of BP3KO mice than in the other strains. ALSKO mice exhibit a significantly lower fraction of BM c-fms+ cells. LID do not differ from Control. Determination of the cellular distribution of B220+ cells by FACS reveal the fraction of these pre-pro-B cells, which express membrane-bound and secreted RANKL, to be significantly lower in ALSKO mice as compared to Control in BM. (B) FACS sorting of BM B220+ cells followed by RT-PCR reveal significant differences for gene expression of RANKL in ALSKO and BP3KO compared to Control. Expression is 4-fold lower in ALSKO and 16-fold greater in BP3KO. *FACS analysis, cell sorting and gene expression*- BM cells were harvested from the femurs of male mice (n=5 mice per group). The cells were washed with phosphate-buffered saline (PBS) and re-suspended in staining buffer (0.5% fetal bovine serum, 0.09% sodium azide). 10^6 cells were preincubated with rat anti-mouse CD16/CD32 (1 µg; BD Biosciences) for 10 min at 4°C to block Fc receptors. Cells were incubated for 30 minutes at 4°C with fluorescently labeled antibodies: R-Phycoerythrin (PE)-c-Fms/CSF-1R (Santa Cruz Biotechnology, Inc), PE-Cy7-conjugated rat anti-mouse CD45R/B220 and Alexa Fluor® 647-conjugated rat anti-mouse CD11b (BD Biosciences). The cells were washed with cold PBS buffer and then resuspended in 100 µl of staining buffer and 10 µl of Streptavidin-FITC 1:100 dilution (BD Biosciences) were added. The cells were incubated for 20 min at 4°C, washed twice with cold staining buffer and resuspended in PBS containing 1% paraformaldehyde. Cell acquisition was performed in a flow cytometer (FACScan, Becton Dickinson), and a minimum of 10,000 events was acquired for each test. Data was analyzed with FlowJo software (version 7.2). R-PE-conjugated mouse IgG_{2b} and Alexa Fluor® 647-conjugated mouse IgG_{2a}, isotype controls were used. Cells were stained with Streptavidin-FITC diluted 1:100 as control. Gene expression based on total RNA was determined by RT-PCR as detailed in Methods. Data are expressed as mean ± SEM. * $p < 0.05$ versus Control.