Figure S1. G-CSF inhibits expression of osteoblast-lineage markers at the endosteum.
qRT-PCR for osteocalcin mRNA in endosteal cells from G-CSF-mobilized mice, and for Runx2, osterix and PTHR1 mRNA in endosteal cells at day 4 of treatment with saline (Sal) or G-CSF (G4). Each dot represents an individual mouse and bars are mean values for each group. * = p<0.05; ** = p< 0.01; *** = p<0.001.
Figure S2. Sorting strategy to prospectively isolate endosteal endothelial cells, MSC, and osteoblasts and its validation
A: Following magnetic depletion of lineage-positive cells, endosteal cells were stained for Lin, CD45, CD31, Sca-1 and CD51. Within the CD45- Lin- gate (top dot plot), endothelial cells were defined as CD31+ cells. Within the remaining CD45- Lin- CD31- cells, MSC were identified as Sca-1+ CD51+, and osteoblasts (OB) as Sca-1- CD51+.
C. Sorted populations were validated by qRT-PCR for endothelial cell specific VE-cadherin, and osteoblast-specific osteocalcin. Expression levels are relative to β2-microglobulin mRNA and are the average of two experiments in which populations were sorted from ten mice each.
B. Repeat experiment in col2.3Cre+ R26R-YFP+ (blue line) and col2.3Cre- R26R-YFP+ negative control mice showed that the osteoblast-specific col2.3 promoter fragment was most active in phenotypic osteoblasts, and inactive in endothelial cells. UD: undetected (>50 cycles).
Figure S3. Endosteal osteoclasts are depleted by clodronate liposome treatment
Tissues were collected from wild-type mice treated with clo-lip or PBS-lip. Histomorphometry for osteoclast surface per bone surface (OcS/BS) and number of osteoclasts per bone perimeter (NOc/BPm) in the trabeculae of the proximal tibial secondary spongiosa from mice treated with clo-lip or PBS-lip. Data are means ± SEM of both tibias from 4-8 mice per group. * = p<0.05; ** = p<0.01; *** = p<0.001. Micrographs are TRAP staining illustrating decrease in osteoclasts (stained red) in clo-lip treated mice versus PBS-lip control mice.