Supplementary Methods

Flow cytometry analysis of peripheral blood, bone marrow and fetal liver cells.

For engraftment and lineage analysis, cells were stained with a cocktail of rat anti-mouse antibodies directed against Ly5.1-PeCy5, Ly5.2-FITC, CD3-PE, B220-PE/APC and Mac 1-APC. For stem cell analysis (Lin-Sca+c-Kit+ and SLAM-markers) of PB and BM antibodies were directed against lineage-positive cells (CD4, CD5, GR1, Lyt2, Ter119, CD11b and CD45R/b220)-PeCy5, c-kit-Alexa 780, Sca1-PE, CD48-APC and CD150-PeCy7 (Becton Dickinson). 7-AAD was added for detection of nonviable cells (Sigma, St Louis, MO). For stem cell analysis of FL antibodies were directed against lineage-positive cells (B220, GR1, Ter119 and CD3)-PeCy5, c-kit-PE, Sca1-FITC, CD48-APC and CD150-PeCy7 (Becton Dickinson). Analysis was performed using a FACS CantoII Instrument (Becton Dickinson, NJ, USA).

Cell cycle analysis

BM cells (oc/oc-R n=5, RANK KO-R n=5 and Ctrl-R n=4) and FL cells (n=4 for each population) were analyzed with respect to cell cycle status. FL cells were c-kit enriched using MACS columns (Miltenyi Biotec). For the cell cycle analysis of BM, antibodies were directed against lineage-positive cells (CD4, CD5, GR1, Lyt2, Ter119, CD11b and CD45R/b220)-PeCy5, c-kit-APC780, Sca1-APC, CD48-FITC, CD150-PeCy7, Ki67-PE and DAPI-Pacific Blue. For the analysis of FL cells the lineage cocktail was (CD3, Ter119, B220, Gr1)-PECy5. All antibodies were from Pharmingen, Becton Dickinson. The cells were fixed using 0.4% paraformaldehyde (Sigma-Aldrich) in PBS for 30 minutes at room temperature, and then incubated with an equal volume of 0.2% Triton-X (Sigma-Aldrich) for one hour at 4°C. The fixed and permeabilized BM cells were washed, resuspended in PBS (Invitrogen), and stained with Ki67-PE (BD Biosciences) for 2 hours. Cells were washed and resuspended in PBS

(Invitrogen) containing 7-AAD (Sigma-Aldrich) for one hour at 4°C.

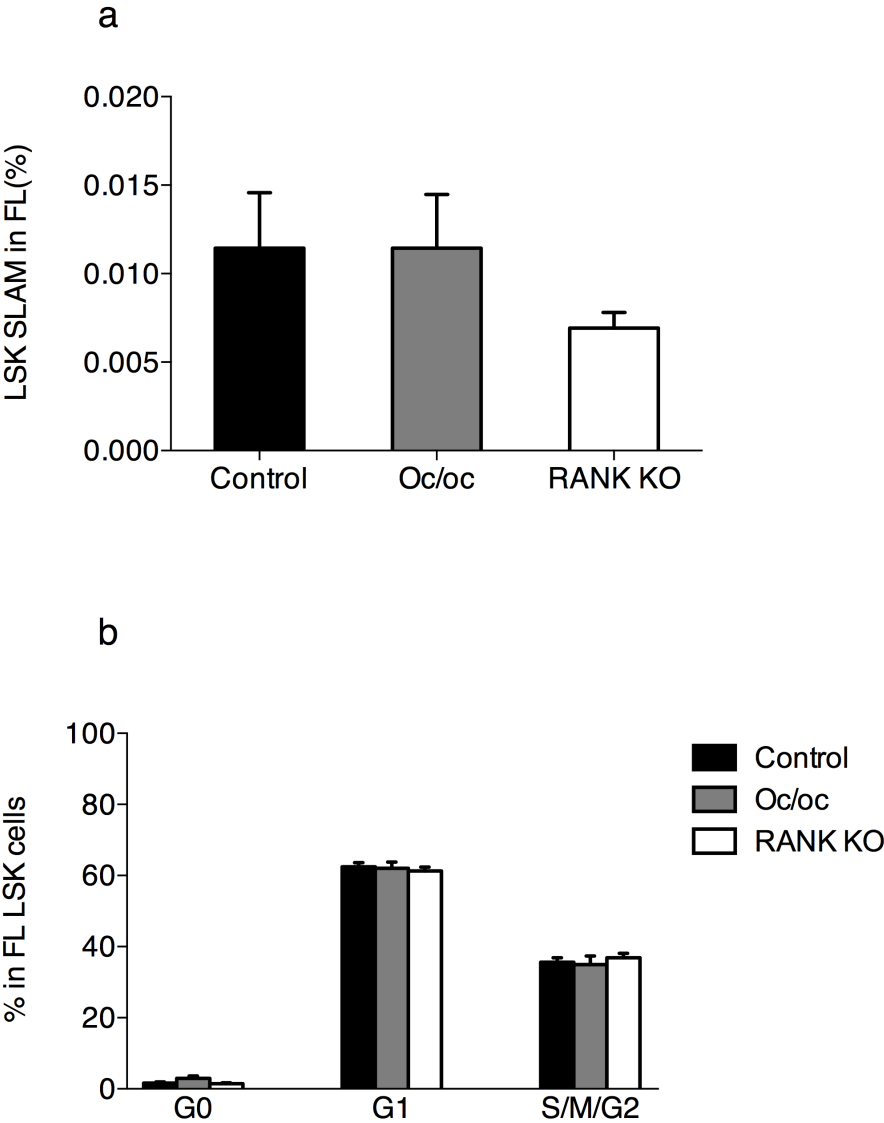
Micro Computed Tomography (µCT)

The cortical and trabecular femoral bone architecture was evaluated using a high resolution µCT scanner (µCT 35; Scanco Medical AG, Brüttisellen, Switzerland). A 232 slices (1392 µm) high region at the mid-femoral diaphysis was scanned in high-resolution mode (1000 projections/180°) with a spatial resolution of 6 µm × 6 µm × 6 µm, an X-ray tube voltage of 55 kVp and current of 145 µA, and an integration time of 600 ms. The 3D data sets were low-pass-filtered using a Gaussian filter ($\sigma = 0.8$, support = 1) and segmented with a fixed threshold filter (628.8 mg HA/cm^3).

Splenocyte osteoclast differentiation and resorption

Isolated spleen cells were differentiated into mature osteoclasts on either bovine bone slices (250000 cells/96-well) or plastic (125000 cells/96-well) by culture in αMEM + M-CSF (25 ng/mL) and mRANKL (100 ng/mL). Media was exchanged every second day, and culture supernatants were collected and stored at -20ºC until further analysis. Resorption pits on the bone slices were visualized by washing them with milli-Q water, followed by removal of remaining cells with a cotton swab and then staining with Mayer's Hematoxylin for 7-8 min, followed by another washing in milli-Q water. Remaining background staining was removed with cotton swab if necessary. The resorbed area was measured using NewCAST software (Visiopharm, Hørsholm, Denmark) with an Olympus IX-70 microscope (Olympus, Center Valley, PA).

Supplementary Figures



Suppl Figure 1. HSC frequency and cell cycle status of the three donor populations. (a) Fetal liver cells were analyzed for the expression of LSK ($Lin^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$) and SLAM ($CD48^{-}$ $CD150^{+}$) markers (n=4). (b) LSK ($Lin^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$) cells from the three donor populations (n=4) were analyzed for cell cycle status by FACS as described in supplementary methods. Data expressed as percentage. Mean values and SEM are shown.