

**Fixation and Immunostaining.** HPC and osteoblast co-cultures were fixed with 4% paraformaldehyde for 15 min at room temperature followed by a 1xPBS wash. Cells were blocked and permeablized in 1xPBS with 10mg/ml of BSA and 0.3% tween-20. Cells were stained at 4°C for approximately 15 h with anti-cd45 (Calbiochem, Gibbstown, NJ), anti-AC133 (prominin-1, Miltenyi Biotec, Germany), anti-CD49d (Calbiochem, Gibbstown, NJ), anti-SARA (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Smad2/3 (BD Biosciences, San Jose, CA) and anti-SDF-1 (Santa Cruz Biotechnology, Santa Cruz, CA). Cells were washed gently with 1xPBS followed by incubation with Alexa-488 goat anti-mouse antibody (Invitrogen, Carlsbad, CA) or Cy3-goat anti-rabbit antibody (Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. Cells were washed following secondary antibody with 1xPBS and mounted in Flouromount-G (Southern Biotech, Birmingham, AL) for imaging.

#### **Scanning Electron Microscopy.**

Cells were rinsed twice with PBS and fixed with 2% glutaraldehyde in 0.1 mol/l Na-cacodylate buffer (with 0.1 mol/l sucrose) at pH 7.4 for 12 h. They were subsequently treated with 1% tannic acid in 0.15 mol/l Na-cacodylate at pH 7.4 for 1 h and post-fixed with 1% osmium tetroxide in 0.1 mol/l Na-cacodylate at pH 7.4 for 1 h. Samples were dehydrated in a graded ethanol series.

**Statistical Analysis.** Error bars are shown as standard deviations and Student *t*-tests were used as a statistical test for all data.

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#### **Supplemental Movies**

Supp. Mov. 1: Time-lapse movie of CD34+ cells co-cultured with GalT-GFP expressing osteoblasts.

Supp. Mov. 2: Time-lapse movie of PKH26 labeled KG1a cells co-cultured with osteoblasts expressing PH-Akt-GFP.

Supp. Mov. 3: Time-lapse movie of N-Rh-PE labeled CD34+CD38- cells co-cultured with primary human osteoblasts.

Supp. Mov. 4: Time-lapse movie of a QD labeled KG1a cell co-cultured with a tubulin-YFP expressing osteoblast.

Supp. Mov. 5: Time-lapse movie of a KG1a cell transiently transfected with CD63-cherry and co-cultured with tubulin-YFP expressing osteoblasts.

Supp. Mov. 6: Time-lapse movie of a CD34+ cell labeled with N-Rh-PE and co-cultured with osteoblasts.