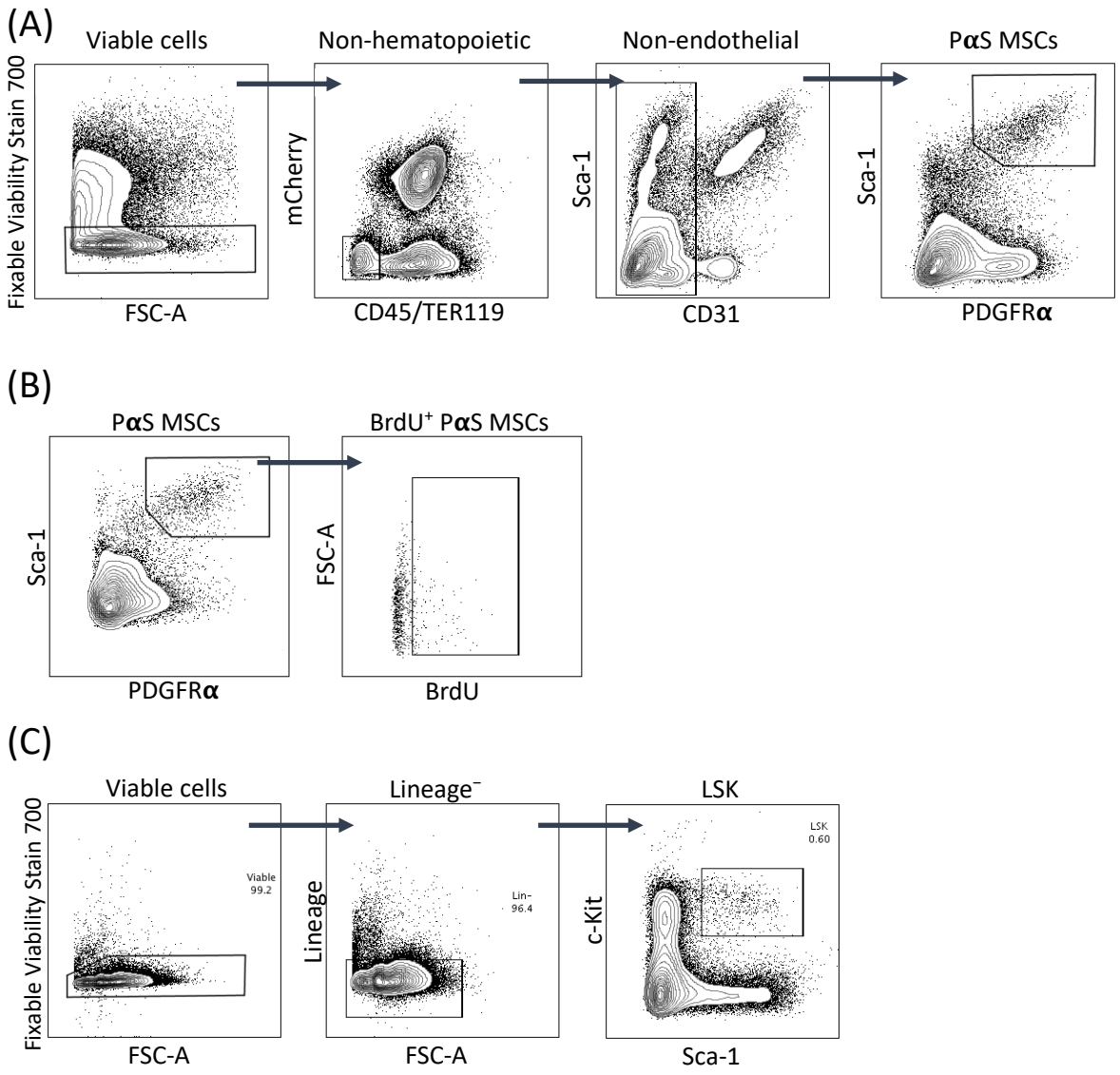

Supplementary Figure 1. Flow cytometry/fluorescence activated cell sorting (FACS) gating strategies. (A) PDGRFα$^+$, Sca-1$^+$ (PαS) mesenchymal stem cell (MSC) gating strategy. **(B)** Detection of BrdU$^+$ PαS MSCs by flow cytometry. **(C)** Lineage$^-$, Sca-1$^+$, c-Kit$^+$ (LSK) gating strategy.

Supplementary Methods

MSC differentiation assays

To assess the osteogenic potential of mesenchymal stem cells (MSCs), MSCs were seeded into a 96-well plate at 8500 cells/well and allowed to adhere overnight. The following day, osteogenic differentiation medium (hMSC Osteogenic Differentiation BulletKit, Lonza) was added to the wells. Cells were cultured for 13-28 days, with media changed every 3 days. After 13 days, cells were washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde (PFA) for 1 minute, followed by washing with a tween wash buffer (0.05% tween in PBS). Differentiating osteoblasts were stained with an alkaline phosphatase solution (SIGMA*FAST* BCIP/NBT tablet, Sigma-Aldrich, dissolved in 10ml of deionized water) for 15 minutes, followed by 2 rinses with the tween wash buffer. To confirm the capacity of mature osteoblasts to form bone nodules, cells were cultured in osteogenic media for 26-28 days. For staining of bone nodules, cells were washed with PBS and fixed with 2.5% glutaraldehyde for 20 minutes. After fixation, cells were washed with PBS once and 70% ethanol thrice. Wells were then air dried, followed by staining with Alizarin Red staining solution (1% in water, pH 4.2) for 20 minutes. After staining, cells were washed twice with 50% ethanol, followed by a third wash in 50% ethanol on a shaking platform. Finally, wells were air dried.

To assess the chondrogenic potential of MSCs, MSCs were resuspended at 45000 cells in 20µl of alpha-MEM medium and seeded into a 48-well plate to form a micromass. Cells were allowed to adhere overnight, followed by culture in chondrogenic differentiation medium (StemXvivo Chondrogenic base media and mouse supplement, R&D). Media was replaced every 3 days for 20-22 days. Following this, each chondrocyte micromass was washed with PBS and fixed with 2.5% glutaraldehyde, followed by staining with an Alcian blue solution (pH=1) to assess glycosaminoglycan content. To make the staining solution, Alcian blue powder (Sigma-Aldrich) was dissolved in 10% sulfuric acid at a concentration of 10mg/ml). After 2 hours, micromass' were washed with a de-staining solution (0.1M hydrochloric acid) followed by PBS twice.

To assess the adipogenic potential of MSCs, MSCs were seeded into a 96-well plate at 15000 cells/well and cultured in complete MesenCult media until confluence. Media was then replaced with Adipogenic Induction Medium (hMSC Adipogenic Differentiation BulletKit, Lonza). After 3 days, media was replaced with Adipogenic Maintenance Medium (BulletKit, Lonza). Three days later, cells were fixed with 4% paraformaldehyde for 1 hour, washed thrice with distilled water and stained with Oil Red O for 30 minutes. The Oil Red O staining solution was made in 99% triethyl phosphate (Sigma-Aldrich) as described previously (Kinkel et al., 2004). After 30 minutes wells were washed thrice with distilled water. Low oxygen culture conditions (5% O_2, 5% CO_2) were maintained throughout adipogenesis (Basciano et al., 2011).

For quantification, 4X images were taken from each well using a Nikon Eclipse Ti system fitted with a Nikon DS-Ri1 color camera. NISElements (V3.21, LO) imaging software was used to capture photos and the percentage of area stained by alkaline phosphatase, Oil Red O or Alcian blue was measured using ImageJ (v1.52a, National Institute of Health) according to a previously published protocol (Ng et al., 2014). For osteoblast mineralization, the number of bone nodules stained by Alizarin Red were counted per well to quantify bone formation.

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