

SUPPLEMENTAL MATERIAL

Morikawa et al., <http://www.jem.org/content/full/jem.20091046/DC1>

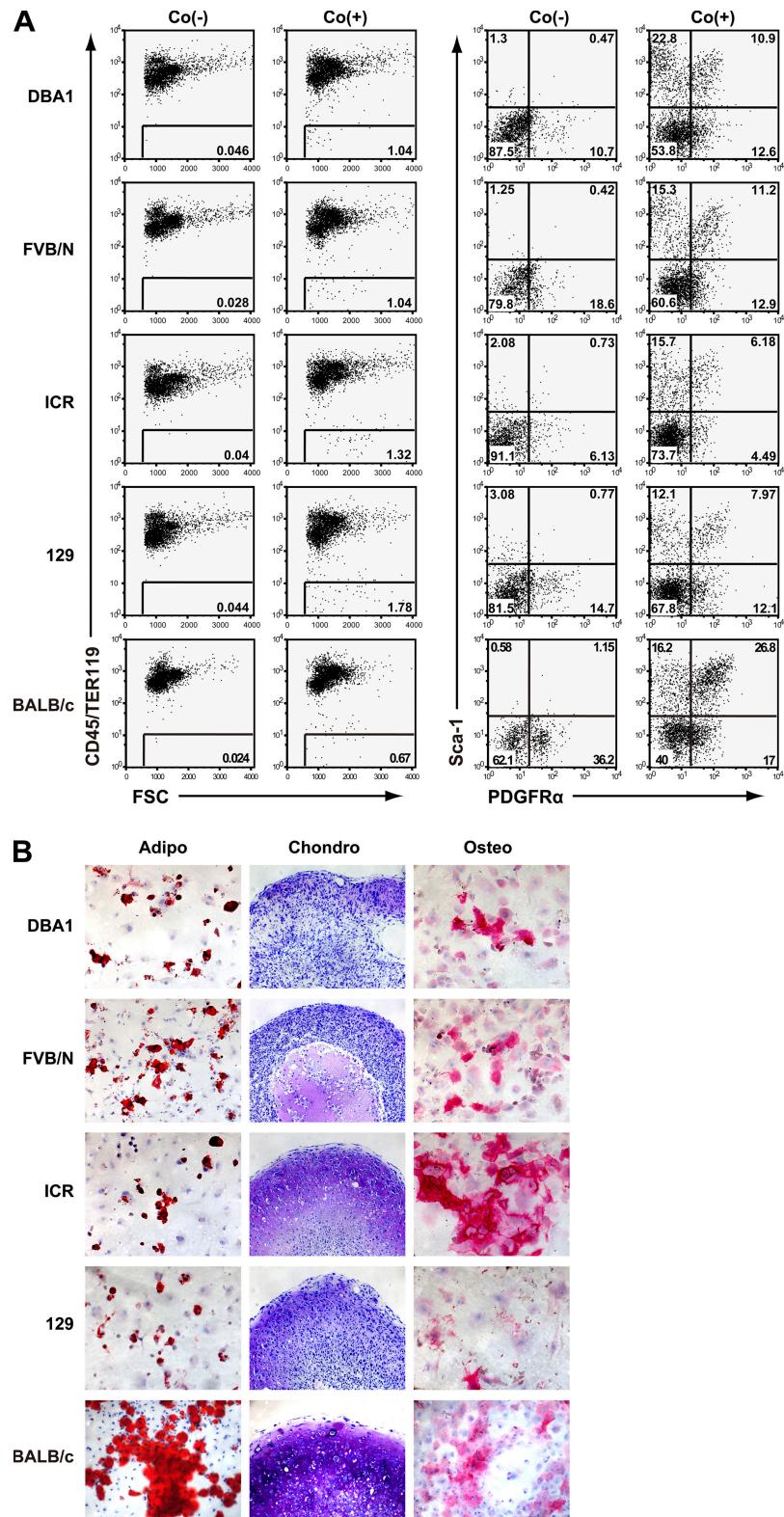


Figure S1. Quantification and differentiation of P α S cells isolated from different inbred mouse strains. (A) The percentage of P α S fraction derived from the BM of four different inbred mouse strains was nearly equal treated with or without collagenase. Similar values were determined for the frequency of CFU-F in cell suspensions obtained from P α S cells of other strain mice, indicating the reproducibility of these values among strains. (B) P α S cells in each strain were incubated to semiconfluence, and then transferred to adipogenic, chondrogenic, and osteogenic medium for 21 d. Bar, 100 μ m. Data are representative of three independent experiments.

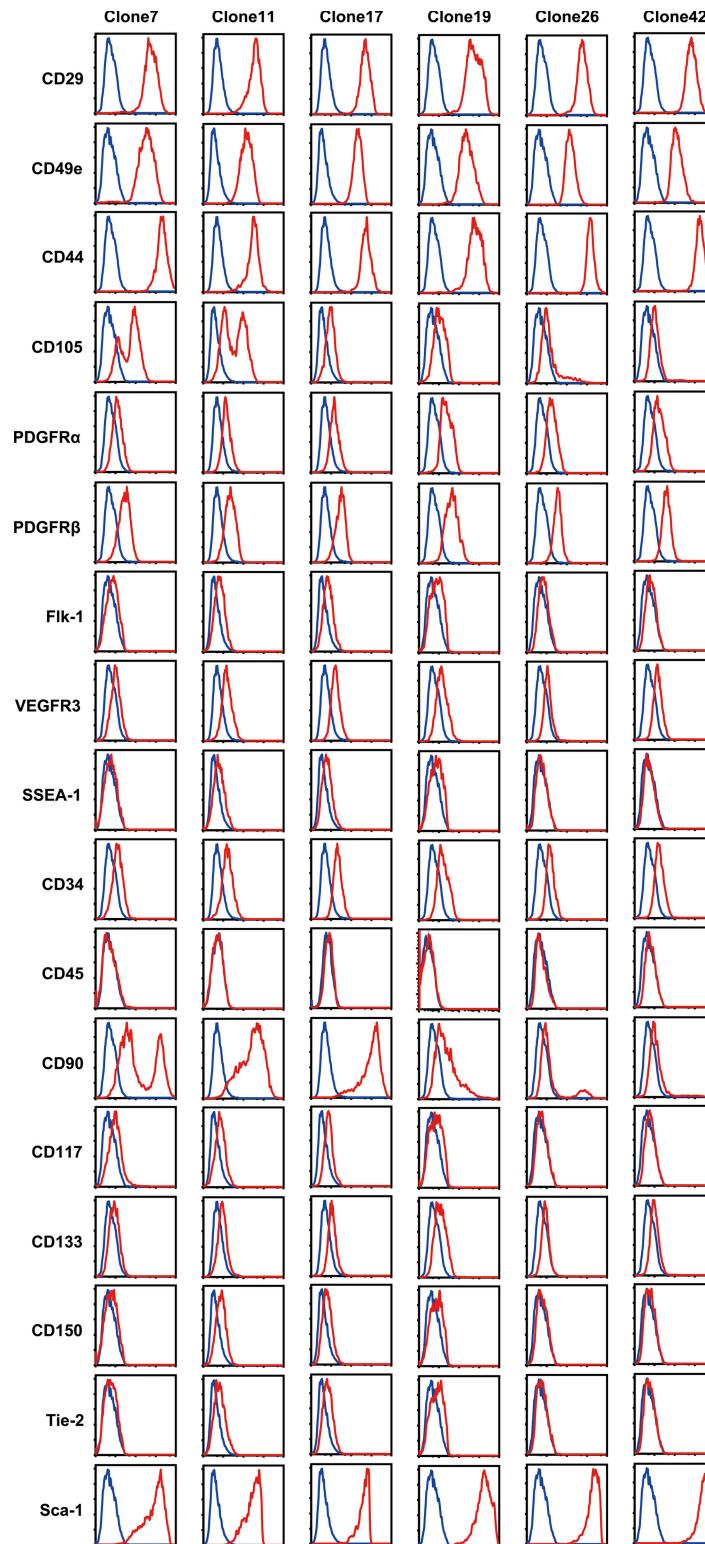


Figure S2. Cell surface marker antigens of clonally cultured PoS cells. Clonal PoS cells cultured for over 100 d were labeled with PE-coupled antibodies against CD49e, Flk-1, Notch-1, SSEA-1, CD45, CD117, CD150, Tie-2, and Sca-1, respectively. CD44 and CD90 were labeled with FITC-coupled antibodies. Biotinylated antibodies against CD29, CD105, PDGFR α , PDGFR β , VEGFR3, CD34, and CD133 were visualized with FITC-conjugated streptavidin. Immunoglobulin isotype control antibodies were prepared as a negative control. Cells were analyzed using FACSCalibur. Blue line, isotype control; red line, specific antibodies. The data revealed the positive, but relatively low, fluorescence intensity of PDGFR α , PDGFR β , endothelial cell markers VEGFR3 and CD34, and CD133 (a marker for various stem cells). No expression of hematopoietic marker CD45, immature ES cell marker SSEA-1, HSCs markers Flk-1, CD117, CD150, or Tie-2.

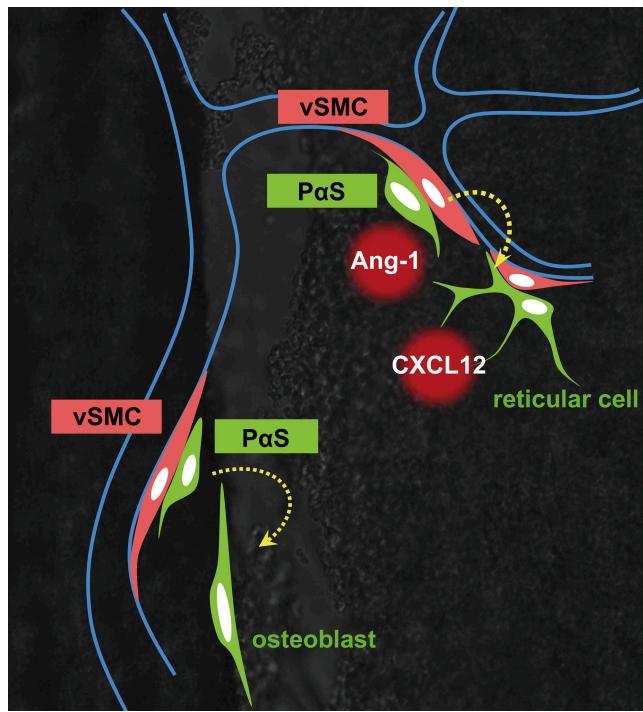


Figure S3. Schematic model of the physiological localization and behavior of MSCs in BM. PaS cells located in the arterial perivascular space in association with vSMCs give rise to some population of osteoblasts and reticular cells functioning as vascular niche cells, which produce major chemoattractant for hematopoietic stem cells.

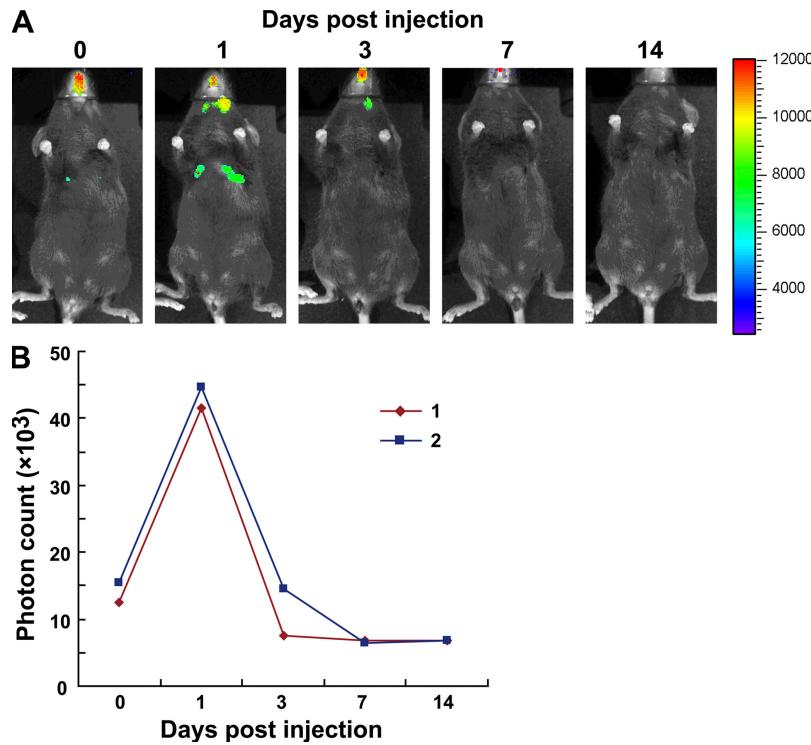


Figure S4. Imaging of transplanted cultured MSCs *in vivo*. (A) Luciferase imaging of cultured MSC migration in the recipient animals at indicated time points after injection of 10^5 cultured PaS cells with 100 CD34-KSL HSCs. (B) Quantitation of luciferase activity in recipient animals. Luminescent intensity was significantly increased after injection of the cultured MSCs in lung and neck regions. However, it dramatically decreased at day 3 and was undetectable after day 7. Data are representative of two independent recipients.

Table S1. Primers used in this study

Gene	Forward	Reverse
<i>Adipsin</i>	5'-ATGGTATGATGTGCAGAGTAGT-3'	5'-CACACATCATGTTAATGGTGAC-3'
<i>PPARγ</i>	5'-AACTGCAGGGTCAAACCTCTGGGAGATTCTCC-3'	5'-GGATTCAAGCAACCATTGGTCAGCTCT-3'
<i>mLP</i>	5'-GAGGACACTTGTCTCATCTCATTTC-3'	5'-CCTCTTATTGGTCAGACTTCC-3'
<i>CollagenII</i>	5'-GGCTTAGGGCAGAGAGAGAAGG-3'	5'-TGGACAGTAGACGGAGGAAAGTC-3'
<i>CollagenX</i>	5'-CAGCAGCATTACGACCCAAG-3'	5'-CCTGAGAAGGACGAGTGGAC-3'
<i>Aggrecan</i>	5'-CCAAGTTCCAGGGTCACTGTTAC-3'	5'-TCCCTCCGGTGGCAAAGAAG-3'
<i>Osteopontin</i>	5'-CAGTGATTGCTTGCCTGTTG-3'	5'-GGTCTCATCAGACTCATCCGAATG-3'
<i>Osteocalcin</i>	5'-GACCATCTTCTGCTCACTCTG-3'	5'-GTGATACCATAGATGCGTTGTAG-3'
<i>PThr</i>	5'-GACAAGCTGCTCAAGGAAGTTCTG-3'	5'-GGAATATCCCACGGTAGATCATG-3'
<i>GAPDH</i>	5'-ACCACAGTCATGCCATCAC-3'	5'-TCCACCAACCTGTTGCTGTA-3'

Table S2. Monoclonal antibodies used in this study

Antigen	Clone	Isotype
PDGFR α	APA5	Rat IgG2a, κ
Sca-1	D7	Rat IgG2a, κ
CD45	30-F11	Rat IgG2b, κ
TER-119	TER-119	Rat IgG2b, κ
CD29	Ha2/5	Armenian hamster IgM, κ
CD34	RAM34	Rat IgG2a, κ
CD44	IM7	Rat IgG2b, κ
CD49e	MER5	Rat IgG2a, κ
CD90	53-2.1	Rat IgG2a, κ
CD105	MJ7/18	Rat IgG2a, κ
c-kit	2B8	Rat IgG2b, κ
CD133	13A4	Rat IgG1, κ
PDGFR β	APB5	Rat IgG2a, κ
CD150	9D1	Rat IgG1
Tie-2	TEK4	Rat IgG1, κ
Flk-1	Avas12a1	Rat IgG2a, κ
VEGFR-3	AFL4	Rat IgG2a, κ
SSEA-1	MC-480	Mouse IgM