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Separation and labeling of CD34$^+$ BM-PCs and c-Kit$^+$ Sca-1$^+$ Lin$^-$ BM-PCs.
For separation of bone marrow progenitor cells (BM-PCs) mice (C57BL/6J or GFP$^+$) were killed by an overdose of pentobarbital and both femura were harvested from each animal. Single cell suspensions of the bone marrow were obtained by flushing the femura with Miltenyi Biotec buffer containing 0,6% CPD-A and 2% FCS using a 21-gauge needle. CD34$^+$ BM-PCs were separated as described previously (Dimmeler, S., A. Aicher, M. Vasa, C. Mildner-Rihm, K. Adler, M. Tiemann, H. Rutten, S. Fichtlscherer, H. Martin, and A.M. Zeiher. 2001. *J. Clin. Invest.* 108:391–397). In brief, the bone marrow cell suspension was incubated with biotin anti–mouse CD34 and immunomagnetic antibiotin microbeads (Miltenyi Biotec) for 30 min at 4°C. Thereafter, cells were washed in PBS containing 0.5% BSA and 0,6% CPD-A, filtered through a 40-μm cell strainer, and run over a magnetic cell separation device (Auto-Macs; Miltenyi Biotec) for positive selection of CD34$^+$ cells. Purity of CD34$^+$ cells was >90%. Primitive c-Kit$^+$ Sca-1$^+$ Lin$^-$ BM-PCs were purified as described previously (Sata, M., A. Saiura, A. Kunisato, A. Tojo, S. Okada, T. Tokuhisa, H. Hirai, M. Makuuchi, Y. Hirata, and R. Nagai. 2002. *Nat. Med.* 8:403–409). In brief, total bone marrow cells were stained with a cocktail of biotinylated monoclonal antibodies against lineage markers (CD5, CD45R, CD11b, TER119, Ly-6G (Gr-1); Stem Cell Technologies) for 15 min at 4 °C. The cells were incubated with Biotin selection kit and magnetic nanoparticles according to the manufacturer's recommendations (Stem Cell Technologies) for 15 min to remove highly lineage-positive cells. The remaining cells were collected and stained with propidium iodide (1 μg/ml; Invitrogen), FITC-conjugated anti-Sca-1 (Ly 6A/E) antibody (BD Biosciences) and PE-conjugated anti–c-Kit (CD117) antibody (BD Biosciences) for 30 min at 4 °C. The cells were analyzed using the high speed MoFlo cytometer (DakoCytomation) and c-Kit$^+$ Sca-1$^+$ cells were sorted. After sorting, the purity of c-Kit$^+$ Sca-1$^+$ Lin$^-$ cells was >98%. For in vivo videofluorescence experiments, CD34$^+$ or c-Kit$^+$ Sca-1$^+$ Lin$^-$ BM-PCs were fluorescently labeled with 5-carboxyfluorescein diacetate succinimidyl ester (DCF). For each experiment, 10^7 fluorescent CD34$^+$ BM-PCs or 1.5 X 10^5 fluorescent c-Kit$^+$ Sca-1$^+$ Lin$^-$ cells were infused intravenously.

Effects of anti-GPIbα mAb on platelet function.
To define the effects of anti-GPIbα mAb on platelet function in vitro, murine platelets were isolated (0.5 X 10^8 cells) and platelet aggregation was induced by 3 μg/ml ristocetin (Probe & Go Labordiagnostica GmbH) or ristocetin plus fibrinogen (10 μg/ml) in the presence or absence of anti-GPIbα mAb (20 μg/ml). Platelet aggregation was determined using a aggregometer (Chrono-Log 500 VS, Probe & Go Labordiagnostica GmbH). To define the effects of anti-GPIbα mAb on platelet adhesion in vivo, carotid injury was induced in anesthetized C57BL6/J mice as described previously (Massberg, S., M. Gawaz, S. Gruner, V. Schulte, I. Konrad, D. Zohlnhofer, U. Heinzmann, and B. Nieswandt. 2003. *J. Exp. Med.* 197:41–49; Moers, A., B. Nieswandt, S. Massberg, N. Wettschureck, S. Gruner, I. Konrad, V. Schulte, B. Aktas, M.P. Gratacap, M.I. Simon, et al. 2003. *Nat. Med.* 9:1418–1422; Massberg, S., I. Konrad, A. Bultmann, C. Schulz, G. Munch, M. Peluso, M. Lorenz, S. Schneider, F. Besta, I. Muller, et al. 2004. *FASEB J.* 18:397–399). After vascular injury, DCF-tagged platelets (375 X 10^5 cells) were infused and visualized using a Zeiss Axiotech microscope (20 x water immersion objective, W 20x/0.5, Carl Zeiss MicroImaging, Inc.) with a 100W HBO mercury lamp for epi-illumination as reported earlier (Massberg, S., M. Gawaz, S. Gruner, V. Schulte, I. Konrad, D. Zohlnhofer, U. Heinzmann, and B. Nieswandt. 2003. *J. Exp. Med.* 197:41–49, Moers, A., B. Nieswandt, S. Massberg, N. Wettschureck, S. Gruner, I. Konrad, V. Schulte, B. Aktas, M.P. Gratacap, M.I. Simon, et al. 2003. *Nat. Med.* 9:1418–1422).

Generation of a SDF-1α/eGFP fusion product.
The coding sequence of SDF-1α (accession NM_199168) was amplified from cDNA derived from human bone marrow using the following gene-specific primers: 5`-GCGGCCGCTAGCGCCACCATGAACGCC AAGGTCGTGGTC-3` and 5`-GCGGCCGGATCCAGCTTGTTTAAAGCTTTCTCCAG-GTAC-3`. The obtained PCR fragment was cloned in frame with the EGFP sequence into the NheI and BamHI restriction sites of the expression vector pEGFP-N1 (BD Clontech) with no intervening in frame stop codons. The fusion protein consisted of the SDF-1α sequence, a seven amino acid residue linker region and the enhanced GFP (fusions to the NH_2 terminus of eGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein in vivo).

Transient transfection and staining of DAMI cells.
DAMI cells were differentiated using 100 nM phorbol-myristate acetate (PMA; Sigma-Aldrich) and transiently transfected using the Effectene Transfection Reagent (QIAGEN) according to the manufacturer's recommendations. In brief, 5 × 10^5 DAMI cells were plated directly onto round 12-mm glass coverslips in 24-well plates the day before transfection. On the day of transfection, 0.25 μg of vector DNA (pSDF-1α /eGFP) were first mixed with DNA condensation buffer to a total volume of 60 μl, then 2 μl of enhancer were added. After incubation at room temperature for 5 min, 6.25 μl of Effectene Transfection Reagent (with a ratio DNA to Effectene reagent of 1:25) were added to the DNA-enhancer mixture, mixed, and incubated for 5 min at room temperature. After 10 min, 350 μl of cell culture medium were added to the transfection complexes. This solution was mixed and added to the 24-well plates containing washed DAMI cells grown onto the glass coverslips. Cells were incubated with the complexes at 37°C and 5% CO_2 for 2 d to allow expression of the fusion protein. 48 h after transfection, the cells were fixed with 2% formaldehyde, washed with PBS, and permeabilized using 0.2 % Triton-X 100. After blocking with 3 % BSA, the cells were stained using anti-vWF polyclonal antibody (Chemicon), Alexa 594-tagged goat anti–rabbit IgG (Invitrogen), anti-GFP (Roche Diagnostics), and Alexa Fluor 488-tagged donkey anti–mouse mAb (Invitrogen), respectively, as indicated. Analysis was performed using a LSM510 META confocal laser microscope (Carl Zeiss MicroImaging, Inc.).

Assessment of platelet binding to BM-PCs by scanning electron microscopy.
CD34$^+$ BM-PCs cells (100 X 10^3 cells/well) were cultivated on coverslips in the absence or presence of platelets (10^8 cells/ml) for 24 h. Thereafter, the coverslips were fixed and examined using a field emission scanning electron microscope (JSM-6300F; Jeol Ltd.).

Generation of mouse megakaryocytes.
CD34$^+$ cells isolated as described in the previous paragraphs were cultured in IMDM medium with stable glutamine (Invitrogen), which was supplemented with 1.5% bovine serum albumin (Sigma-Aldrich), 300 μg/mL iron-saturated transferrin, 1 mmol/L sodium pyruvate, 1X minimum essential medium vitamins, 1X minimum essential medium nonessential amino acids, 0.02 mg/mL L-aspargine, 0.01 mmol/L monothioglycerol (all obtained from Invitrogen). Our

new cytokine combination included 10 ng/mL mouse thrombopoietin (TPO) and 10 ng/mL IL-6, both obtained from Cell Systems, 10 ng/mL IL-1β, and 50 ng/mL of stem cell factor (SCF), both obtained from R&D Systems.

Effect of platelet SDF-1α on progenitor cell migration.
Progenitor cells isolated from E7.5 mouse embryos as described previously (Hatzopoulos, A.K., J. Folkman, E. Vasile, G.K. Eiselen, and R.D. Rosenberg. 1998. *Development*. 125:1457–1468) were grown to confluence in six-well plates. The PC monolayers were wounded in a linear fashion with a pipette tip. The wounded PC monolayers were further incubated with platelets (10^8 cells/ml medium) or platelet-free medium for 24 h in the absence or presence of 5 μg/ml anti–SDF-1α (R&D Systems), anti-CD11b (BD Biosciences), or isotype-matched control IgG (BD Biosciences) as indicated. Finally, the number of PCs that migrated into the denuded area was evaluated.

Recruitment of endogenous PCs to the injured carotid artery.
To determine recruitment of circulating progenitor cells, carotid arteries of C57BL/6J ($n = 3$), P-selectin– ($n = 4$), or GPIIb-deficient mice ($n = 2$) or in anti–SDF-1α mAb-treated WT mice ($n = 2$) were injured and *c-Kit* and *Sca-1* mRNA expression was assessed by PCR. In brief, 24 h after endothelial denudation, total RNA was isolated from the injured (right) and the uninjured (left, control) carotid arteries using the RNeasy Micro Kit (QIAGEN) according to the manufacturer's instructions. Contaminating genomic DNA was removed with an on-column DNase I digest using the QIAGEN RNase-free DNase Set. RT-PCR was performed using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). 100 ng of total RNA were reverse transcribed using $Oligo(dT)_{12-18}$ primers and the SuperScript II Reverse Transcriptase according to the manufacturer's recommendations. After reverse transcription, 2 μl of the first-strand cDNA were subjected to PCR amplification. PCR was performed in a 25-μl reaction mixture containing 2.5 U *Thermus aquaticus* (Taq) *polymerase*, 200 μM dNTP's (Invitrogen), 7.5 pmol sense and antisense oligonucleotides (MWG Biotech AG). The reaction mixture was subjected to denaturation for 1 min at 94°C and amplified by 30–38 cycles as follows: denaturation for 30 s at 94°C, annealing for 30 s at the indicated temperature, extension at 72°C for 30 s, with a final 7-min extension at 72° C in a GeneAmp PCR System 9700 Thermocycler (Perkin Elmer). The primer sequences and their corresponding product sizes and annealing temperatures are shown in the supplemental table. The number of PCR cycles in respective amplifications was determined experimentally so that semiquantitative comparisons could be made during the exponential phase of the amplification process. The RT-PCR amplification products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized by UV light illumination. Gel images were captured and densitometric analysis was performed using the GS-800 calibrated densitometer and the image analysis software Quantity One, Version 4.4.1 (Bio-Rad Laboratories). The constitutively expressed β-actin transcript was amplified as an internal control to compare relative abundance of PCR products, and the relative expression of each mRNA was normalized to the expression of β-actin for semiquantification.