Heparan sulfate mimetics can efficiently mobilize long-term hematopoietic stem cells

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Online Supplementary Design and Methods

Flow cytometry

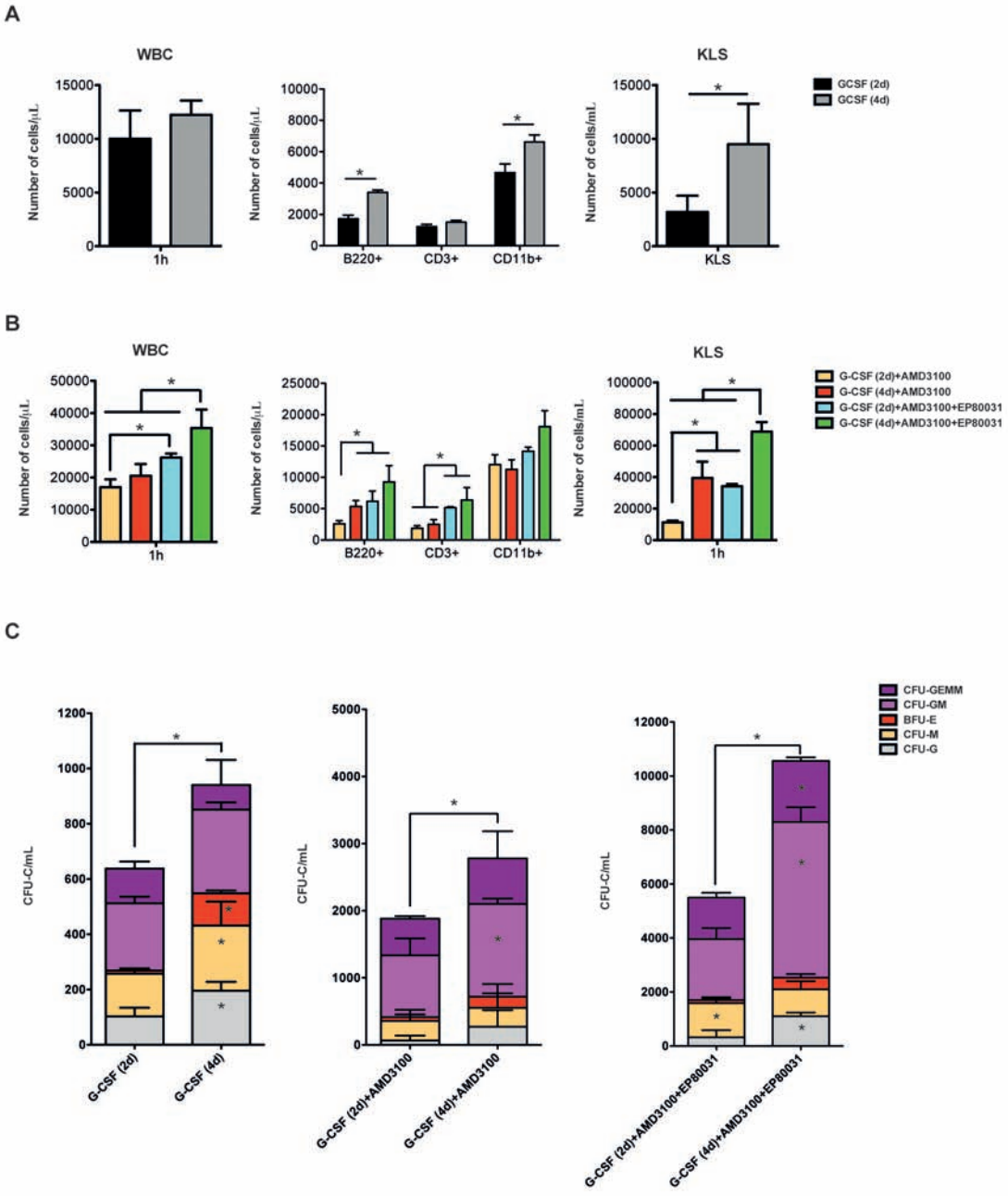
Blood leukocytes and bone marrow cells were stained with antibodies conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), phycoerythrin-cyanine 7 (PE-Cy 7) or allophycocyanin (APC) [all products were from Becton Dickinson Biosciences Pharmingen (BD), San Diego, CA, USA]: CD45.1 (A20), CD45.2 (104), B220 (RA3-6B2), CD3 (145-2C11), CD11b (M1/70), Gr-1 (RB6-8C5), cKit (2B8), Sca1 (E13-161.7). Lineage cells were labeled using a biotin-conjugated lineage cocktail (Miltenyi Biotec, Bergisch Gladbach, Germany). Biotinylated antibodies were revealed with streptavidin- PE-Cy 7 (BD). Stained cells were then analyzed using a FACSCalibur cytometer and CellQuest software (BD).

Quantification of vascular endothelial growth factor and stromal cell-derived factor-1

Mice were intravenously injected with EP80006 or EP80031 at the final concentration of 15 mg/kg. Blood samples were collected 30 min and 1, 3, and 5 h after injections. The blood samples were then allowed to clot for 2 h at room temperature and sera were collected and stored at -20°C until the assay. For vascular endothelial growth factor (VEGF) and stromal cell-derived factor-α (SDF-1α) immunoassays, samples were diluted 1:5 and 1:10 in calibrator diluent RD5T and RD6Q (Quantikine assays), respectively (250 μL final volume). Mouse VEGF and SDF-1α standard concentrations were prepared by 1:2 dilution series from 500 to 7.8 pg/mL (VEGF) and 10 to 0.156 ng/mL (SDF-1α). Fifty microliters of standard, control and diluted samples were incubated on microplate wells in duplicate for 2 h at room temperature, aspirated and washed five times with 400 μL of wash buffer. Then, 100 μL of VEGF or SDF-1α conjugate were added to each well and incubated for 2 h at room temperature, followed by five washes. One hundred microliters (VEGF) or 200 μL (SDF-1α) of substrate solutions were added to each well and incubated for 30 min at room temperature. Reactions were stopped by addition of 100 μL (VEGF) or 50 μL (SDF-1α) of stop solution. Optical densities were measured using a Genios Pro TECAN microplate reader (Tecan Group Ltd. Männedorf, Switzerland) at 450 nm and corrected (subtraction) by reading at 570 nm. VEGF and SDF-1α concentrations in samples were determined using the respective standard curves obtained from known substrate concentrations (standards).

Colony-forming unit assay

Fifty microliters of NH_4Cl lysed blood from mice mobilized with granulocyte colony-stimulating factor (G-CSF), AMD3100, EP80031, G-CSF + EP80031, G-CSF + AMD3100 or G-CSF + AMD3100 + EP80031 were added to 2 mL of complete Methocult and seeded in 35 mm culture dishes. Culture dishes were transferred into an incubator at 37°C, in a 5% CO_2 atmosphere with 95% humidity. The CFU were enumerated after 7 days in culture. Cultures were evaluated for the presence of myeloid and multi-potential CFU and erythroid burst-forming units (BFU-E). Myeloid CFU include colony-forming unit-granulocyte (CFU-G), colony-forming unit-macrophage (CFU-M) and colony-forming unit-granulocyte, macrophages (CFU-GM). Multi-potential CFU include colony-forming units with mixed populations of erythroid and myeloid cells (CFU-GEMM).



Online Supplementary Figure S1. (A) Differential mobilization after 2 days or 4 days of treatment with G-CSF. One hour after the last injection of G-CSF, blood was collected and leukocytes were counted (left panel) and B220-positive B cells, CD3-positive T cells, CD11b-positive granulocytes and monocytes (middle panel) and KLS cells (right panel) present in PB were quantified by FACS analysis. Data are expressed as cell number/μL or cell number/mL (for KLS cells) (mean±SEM, n=6, **P*<0.05). (B) Effect of AMD3100 and EP80031 treatment after 2 days or 4 days of treatment with G-CSF. One hour after the injection of AMD3100 and/or EP80031, blood was collected and leukocytes were counted (left panel) and B220-positive B-cells, CD3-positive T cells, CD11b-positive granulocytes and monocytes (middle panel) and KLS cells (right panel) present in PB were quantified by FACS analysis. Data are expressed as cell number/μL or cell number/mL (for KLS cells) (mean±SEM, n=6, **P*<0.05). (C) Clonogenic capacity of mobilized hematopoietic cells after 2 days or 4 days of treatment with G-CSF alone or in combination with AMD3100 and EP80031. CFU assays were done with PB cells 1 h after the last injection of G-CSF or AMD3100 (double combination) or EP80031 (triple combination). The bar graphs show the number of CFU-G, CFU-M, BFU-E, CFU-GMs and CFU-GEMM. Data are expressed as CFU-C/mL of blood (mean±SEM, n=5, **P*<0.05).