SUPPLEMENTARY INFORMATION, Data S1

MATERIALS AND METHODS

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3 **Peripheral blood sample and generation of Blood-Born Hematospheres (BBHSs)**

4 All study protocols in this study were approved by the Institutional Review Board of Seoul
5 National University Hospital. Fresh peripheral blood (PB) was obtained from healthy donors
6 (mean age 28.2 ± 4.9) with informed consent. Donors with mobilizing condition were screen
7 based on history, systemic review and physical examination by medical doctor (Supplementary
8 Table 1). Human peripheral blood mononuclear cells (MNCs) were isolated by Ficoll-Paque™
9 PLUS (GE healthcare) according to manufacturer's instructions and were washed five times
10 with phosphate buffered saline (PBS). For culture of BBHSs, MNCs were resuspended at 3-
11 5x10^6 cells/ml in EBM-2 with 5%FBS and seeded on Hydrocell™ Ultra-Low attach surface
12 (NUNC). 1ml of EBM-2 with 5%FBS fresh medium was added every third day without media
13 change. BBHSs were followed-up every day.

14

15 **Gene expression analysis**

16 Total RNA was extracted from fresh MNCs or cultured BBHSs using TRIZOL reagent
17 (Invitrogen) according to manufacturer's instruction. RT-PCR was performed as described
18 previously. cDNA was synthesized using a Primescript 1st strand cDNA synthesis kit

(TAKARA) and oligo-dT primer. Primer for human collagen I (P155768), collagen IV (P105000), human Fibronectin (P142696) and human laminin (P153523) were purchased from Bioneer (http://bioneer.co.kr). Information for other primers is provided in Supplementary table 2.

Fluorescent Activated Cell Sorter (FACS) analysis

Flow cytometry was performed as described previously. Fresh MNCs were prepared as above. Cultured BBHSs were treated with 0.5mg/ml dispase (Gibco) and 0.125% Trypsin-EDTA for 5 min at 37°C to dissociate into single cells and washed with PBS. Cells from xenotransplanted NOG/SCID mice are described below. Cells were stained with antibodies specific for cell surface markers and analyzed by flow cytometry using a BD Canto II ™ (Becton Dickinson). FITC-labeled antibody for lineage (Lin) was CD3, CD14, CD16, CD19, CD20 and CD56 (BD Pharmingen). PE-labeled antibodies were human CD38, mouse CD45 (eBioscience). APC-Cy7-labeled antibody was human CD45 (BD Pharmingen). Allophycocyanin (APC)-labeled antibody was human CD34 (BD Pharmingen). The appropriate isotype antibodies served as negative controls.

Whole mount Immunostaining of BBHS

For immunocytochemistry, cultured BBHSs were fixed in 2% cold paraformaldehyde for 10 min

on ice and washed with PBS-T twice. As stem/progenitor markers, we used antibodies against CD34 (1:200; BD Pharmingen). As extracellular components markers, we used antibodies against collagen I (1:400; Southern Biotech), collagen III (1:400; Southern Biotech), collagen IV (1:400; Southern Biotech), fibronectin (1:500; BD Pharmingen) and laminin (1:500; Abcam). As proliferation markers, we used antibody against Ki-67 (1:400; Abcam). Integrin αVβ1 was stained as fibronectin receptor (1:200; Chemicon). To define hematopoietic population in BBHS we used CD14 (1:200, BD Pharmingen) and CD45 (1:200, BD Pharmingen). To visualize SDF-1-CXCR4 axis we used SDF-1 (1:200, R&D Systems) and CXCR4 (1:200, eBiosciences).

Colony-Forming-Unit Assay of BBHSs

Colony-forming unit (CFU) assay was performed using MethoCult GF H4434 (Stem Cell Technologies, Vancouver). 1 x 10^5 freshly isolated MNC or its equivalent of single-suspended BBHS cells were plated in triplicate and cultured for 14 days at 37°C with 5% carbon dioxide in a humidified atmosphere. CFU-E were numerated on day 7 and BFU-E, CFU-GM and CFU-GEMM were numerated on day 14 of the assay. Colonies were visualized under inverted microscope, classified according to their morphological characteristics and counted.

In vivo Bone Marrow (BM) Repopulation of BBHS derived HSPCs

Xenotransplantation of the BBHSs was performed by modification of the method previously described. Briefly, 8-to-10 week old severe immunocompromised NOD/Shi-scid IL2Rg^{null} (NOG-SCID) mice were given 240 rads of whole body irradiation to ablate the hematopoietic system partially. 5 x 10^4 single-suspended BBHS (Day 5 to 7) cells were injected through intracardiac puncture. Mice were anesthetized and killed by cervical dislocation 12 weeks after transplantation. To assess hematopoietic chimerism, Peripheral blood (PB) was collected by puncture of the left ventricle of the heart. Femurs, tibiae and humeri were collected and flushed with PBS to liberate BM cells. Flushed BM aspirates were then filtered through a sterile 40-μm cell strainer (BD Falcon) to get rid of clumps and debris. Spleens from mice were gently smashed by pressing with the flat surface of a syringe plunger, collected and filtered through a sterile 40-μm cell strainer. After RBCs were lysed, leukocytes were processed for flow cytometric analysis. For secondary bone marrow repopulating experiment, whole chimeric bone marrow cells were tagged with mouse hematopoietic lineage / HSPC markers (PE-Ly6G, CD90.2, CD49b, CD45R/B220, CD45, c-kit, Prominin I and Sca-1 antibody) and human lineage markers (FITC-CD3, CD4, CD8, CD14, CD19 and CD56). Subsequently, anti-FITC and anti-PE magnetic beads were used to deplete the whole chimeric bone marrow of mouse cells and human non-HSPCs. The negative fraction was washed twice with PBS. Total 5x10^4 cells of the human HSPC-enriched negative fraction were injected into NOG-SCID mice.

Image acquisition

Images were acquired using an Olympus IX2 inverted fluorescence microscope (Olympus, Tokyo, Japan) equipped with an Olympus DP50 CF CCD camera and analySIS 5.0 software. Confocal images were acquired using Zeiss LSM-710 META confocal microscope and ZEN 2008 analysis software.

Statistical analysis

Data are expressed as means ± S.E.M. The statistical significance of difference between two groups was evaluated with an unpaired t-test and the significance between three groups was analyzed with one-way analysis of variance (ANOVA) followed by Bonferroni's method. Probability values less than 5% were considered significant. All calculations were performed using SPSS 12.0.

Supplementary information, Table S2

primer	Sequence	Tm (℃)	Access Number	Product size (bp)
Col3A1	Forward 5'- CCCACTATTATTTTGGCACAACAG -3' Reverse 5'- GCATGGTTCTGGCTTCCAGA -3'	62	NM_000090.3	244
ITGAV (Integrin αV)	Forward 5'- TTGTTGCTACTGGCTGTTTTG -3' Reverse 5'- TCCCTTTCTTGTTCTTCTTGAG -3'	57	NM_002210.3	469
ITGB1 (Integrin β1)	Forward 5'- GAACGGCCCCTGACACCTGC -3' Reverse 5'- GCACCACCCACAATTTGGCCC -3'	62	NM_133376.4	290
GAPDH	Forward 5'- CAAATTCGTTGTCATACCAG -3' Reverse 5'- CGTGGAAGGACTCATGAC -3'	60	NM_002046.3	504
SDF-1a	Forward 5'- GCCGCACTTTCACTCTCCGT -3' Reverse 5'- TTTCCTCGAGTGGGTCTAGCGGA -3'	60	NM_199168.3	472
CXCR4	Forward 5'- TACCCACACGGTGTTGTGAGAATGA -3' Reverse 5'- CCGCGCTTTCAGCAGACGCA -3'	65.5	NM_001008540	449

Table S2 Primer sequences, Tms, and the sizes of PCR products