FACS analysis of CB_2 expression in human mononuclear cells and human HSCs
Mononuclear cells were washed, resuspended in PBS+ (PBS containing 2% FBS) and blocked with Fc blocking reagent (Miltenyi Biotec). After blocking, the cells were incubated on ice with polyclonal antibodies against CB_2 (Affinity BioReagents) for 30 min, followed by staining with FITC-conjugated or APC-conjugated secondary goat anti-rabbit antibody (Jackson Immunoresearch Laboratories). For the double staining, the cells were further stained for 30 min on ice with PE-conjugated or FITC-conjugated anti-CD34 (Pharmingen) or anti-CD38 (Pharmingen) antibodies. Human $CD34^+$ cells were isolated from the mononuclear cells by using magnetic beads from a kit obtained from Miltenyi Biotec according to the manufacturer's instructions. The cell surface expression of the above molecules was examined by flow cytometry analysis using a FACSCalibur flow cytometer (Becton Dickinson) or a dual-laser–Mo-Flo high performance cell sorter (Cytomation, Inc., Fort Collins, CO).

Preparation of mouse bone marrow cells, FACS and Hoechst 33342 staining
Murine bone marrow cells were harvested from C57BL/6J mice by flushing the femurs and tibias, and then placed in $DMEM^+$ (Dulbecco's modified Eagle's medium, 2% FBS, 10 mM HEPES buffer, Gibco, Grand Island, NY, USA). A single cell suspension was made in $DMEM^+$ by passing the bone marrow through an 18-gauge needle. The cells were filtered through 70-micron cell strainers (Falcon) and pelleted by centrifugation. The cells were resuspended in prewarmed $DMEM^+$, and viable cells were counted using the trypan blue dye exclusion method. Bone marrow cells were stained with Hoechst 33342 (Sigma), as described previously (49). The Hoechst-stained cells were separated using Percoll reagent (Amersham-Pharmacia, Uppsala, Sweden) to remove the red blood cells as well as the dead cells. The Hoechst-stained and separated cells were then suspended in $HBSS^+$ (Hank's balanced salt solution, containing 2% FBS and 10 mM HEPES buffer, Gibco) at 20 to 30 $\times 10^6$ cells per ml cell density for the antibody staining. Immunostaining with anti-CB_2 antibodies was followed by FACS analysis as described above, except that PE-conjugated secondary antibodies were used. The flow-cytometric analysis to determine Hoechst and PE fluorescence was performed using a dual-laser–Mo-Flo high performance cell sorter (Cytomation, Inc., Fort Collins, CO), as described previously (*20, 49*). FACS analysis of murine bone marrow was performed using: $B220^-$, $CD3^-$, $Gr1^-$, $CD19^-$, $Ter119^-$, mac1 (cychrome conjugated), c-kit (APC), sca-1 (PE) antibodies, CD48 biotinylated (followed by a streptavidin PE-Texas red conjugated), CD150 (PE or FITC), CXCR4-PE and VLA4-FITC, (all antibodies were from BD biosciences). For murine SLAM/LSK isolation, we used Sca-1-PE-Cy7, c-kit–FITC, CD150 APC, CD48− biotin and streptavidin-PE, as described (27, 29, 50).

RT-PCR analysis of CB_2 expression
RNA from total bone marrow MNCs as well as isolated $CD34^+$ and $CD133^+$ cells was extracted using the RNeasy Mini Kit along with DNA Shredder Kit (both from Qiagen, Valencia, CA) following the manufacturer's protocol. A QIA shredder spin column and DNase I digestion were included in the isolation procedure to limit the possibility of PCR amplification of *Cnr1* and *Cnr2* from genomic DNA. cDNA and PCR amplification were performed with the BD Biosciences TITANIUM One-Step RT-PCR Kit using 200 ng of RNA as a template for first-strand synthesis. *Cnr2* was amplified using: 5'-CCA TGG AGG AAT GCT GGG TG-3' and 5'-TCA GCA ATC AGA GAG GTC TAG-3', which yield a product of 1100 bp. GAPDH was used as a positive control with primers: 5'-CTC ACT GGC ATG GCC TTC CG-3' and 5'-ACC ACC

CTG TTG CTG TAG CC-3', which yield a product of 292 bp. The template was first denatured at 94°C for 2 min followed by 35 cycles (94°C for 30 sec, 58°C for 30 sec and 68°C for 1 min), followed by 68°C for 2 min in a myCycler Personal Thermal Cycler (Bio-Rad Laboratories, Inc). Aliquots (20 µl) of the PCR products were run on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide.

Western blotting

For the Western blotting procedure, isolated $CD34^+$ cells (1×10^4) derived from bone marrow were purchased from Cambrex. The procedure was performed using protein total cell lysates of $CD34^+$, human bone marrow cells and Jurkat cells (1×10^4) as a positive control. Total protein was extracted in lysis buffer containing 20mM Tris HCL (pH7.5), 150 mM Nacl, 10mM NaF, 1% Nonidet P-40, 10% glyceriol, 1mM PMSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, and 0.5 mM Na_3VO_4. Proteins (40 µg) were analyzed through poly-acrylamide-SDS gels and transferred by electroblotting onto nitrocellulose membranes. Membranes were blocked for one hour in 5% BSA before being incubated with appropriate dilutions of primary phospho-specific antibody as well as corresponding secondary antibody. Blots were developed using the ECL system (Immobilon, Western, Millipore). Membranes were then stripped, reblocked, and reprobed to detect total actin or Rac1 or as indicated. Immunoblots were scanned, and quantification was carried out by Image Quant software version 3.3 (Molecular Dynamics).

Rac1-GTPase pull-down assay

Rac1 activation assay kit was purchased from Cytoskeleton, and assays were performed according to attached protocols. In brief, 1×10^7 human MNC pretreated with PTX or vehicle: 0.01% DMSO in IMDM medium) were stimulated with 1 µM AM1241 or vehicle for 4 min and immediately lysed with lysis buffer supplied in the kit and centrifuged at 14,000*g* for 3 min. A portion of each supernatant was diluted in SDS-PAGE sample buffer for detection of total (both GTP- and GDP-bound) Rac1 GTPases. The remaining supernatant fractions were incubated with 20 µl of PAK-PBD agarose for 1 h at 4°C, followed by washing twice with wash buffer. Proteins bound to the beads were eluted in 20 µl of 2×SDS-PAGE sample buffer, and they were subjected to Western blot analysis.

Endocannabinoid levels in bone marrow stromal cells

Murine bone marrow stromal cells (10^7) either control or following exposure to the stress inducer LPS at 1ng/0.1ml for 24 hrs. Cells were then analyzed for endocannabinoid expression level. Mixtures of the 2-AG and AEA and their denatured analogs that had been stored at −80°c were further diluted in a 20mg/ml solution of fatty acid free bovine serum albumin (BSA). The calibration standards, quality control (QC) samples and reference samples were analyzed as previously described (20, 51). The calibration curves were constructed from the ratios of the peak areas of the analytes versus the IS.

LC-MS analysis for endocannabinoids

Chromatographic separation was achieved using a Higgins Analytic Haisil C18 column (0.5 × 50mm, 5mm) on an ABI 4000 Q-Trap mass spectrometer with a Tempo nano-LC on the front end (Applied Biosystems Incorporated; Framingham, MA). The mobile phase consisted of 95/5 water/acetontrile and 95/5 acetonitrile/water, with 0.1% formic acid in both, in the following gradient: initial conditions are held at 30% A for 30 seconds, increased linearly to 100% B and

held from 0.75 to 4 min, then returning to initial conditions by 4.5 minutes (flow rate=10 μL/min); the autosampler was kept at 4°C to prevent analyte degradation. Eluted peaks were ionized via electrospray ionization (ESI) in MRM mode.

Live cell binding assay

$1.5–2.5 \times 10^5$ MDA-MB-231 cells or 293T cells were plated in 96 well puncher plates in full media (6 rows down and 8 rows across) and grown to reach a density of 75%. To assign absolute affinity of each ligand for CXCR4 receptor, a competitive displacement assay was employed (36) using cold ligand SDF-1α or CB2 as test compounds, along with [^{99m}Tc-MAS$_3$]SDF1α as radiotracer on the surface of MDA-MB-231 cells (CXCR4 positive) and 293T (CXCR4, negative) cell lines [36, 37]. To avoid internalization of the radioligand due to constitutive endocytosis, live cell binding was performed at 4 C. Cells were washed 2 times with ice-cold phosphate buffer (PBS), pH 7.4 and incubated for 20 min at 4 C with 0.5 μCi of radiotracer in the presence or absence of the test compound. Cells were then washed 3 times with PBS and the well contents transferred directly to 12 × 75 mm plastic tubes placed in gamma counter racks. Transfer was accomplished using a modified (Microvideo Instruments, Avon, MA) 96-well puncher (Millipore MAMP09608) and disposable punch tips (Millipore MADP19650). Well contents were counted on a model 1470 Wallac Wizard (Perkin Elmer, Wellesley, MA) ten-detector gamma counter.

Table S1. Effects of AM1241 on WBC, RBC, platelets, and neutrophils

	WT	AM1241 Treated
WBC (1×10^3 cells /ul)	6.99 ± 0.23	6.31 ± 0.41
PLT (1×10^6 cells /ul)	15.86 ± 2.16	12.56 ±1.76
RBC (1×10^6 cells /ul)	9.5 ± 0.31	9.87 ± 0.43
Neutrophills (1×10^6 cells /ul)	1.32 ± 0.33	1.41 ± 0.37

Hematologic evaluation of white blood cells (WBC), red blood cells (RBC), platelets (PLT) and neutrophills. Values are mean ± s.d. (N=16). Mice administered with either vehicle control or with AM1241 once daily i.p. for 4 days. At day 5, peripheral blood were obtained and analyzed for hematologic evaluation of mouse blood. A representative experiment out of 3 experiments.

Table S2. Mobilization of hematopoietic progenitor subsets after treatment with AM1241 and G-CSF

Progenitor Subsets	Mobilization by:		
	Vehicle	AM-1241	G-CSF
CFU-GM	157 ± 25	1432 ± 121*	1703 ± 195*
BFU-E	16 ± 3	142 ± 17*	165 ± 31*
CFU-GEMM	13 ± 4	49 ± 6*	66 ± 24*

Mice were admistered with either vehicle, AM1241 (i.p. once daily for 4 days 10mg/kg) or G-CSF twice daily i.p. for 4 days. Blood samples were collected after 24 hours and analyzed for progenitor subtypes as detailed in "Materials and Methods". Data are presented as the mean ± SEM per ml blood of 3 experiments. $p<0.05$ compared with vehicle control.
AM1241-CB2 agonist; G-CSF–granulocyte colony-stimulating factor; CFU, colony forming unit; GM, granulocyte-macrophage; BFU-E–, burst forming unit erythrocyte; GEMM, granulocyte-erythrocyte-monocyte-megakaryocyte.

Table S3. Flow cytometric analysis of peripheral blood (6 and 20 weeks) and bone marrow (20 weeks) after transplantation

Group	Animal #	% Ly5.2 + cells			Tri-lineage reconstitution
		Blood 6 weeks	Blood 20 weeks	BM 20 weeks	
Non-mobilized	1	3.8	2.8	0.4	No
	2	3.2	2.5	0.4	No
	3	1.6	1.2	0.2	No
	4	3.5	2.2	0.4	No
	5	3.4	1.9	0.3	No
	6	2.5	2.5	0.4	No
	7	3.7	2.4	0.3	No
	8	3.3	2.6	0.6	No
	9	3.5	1.1	0.3	No
G-CSF–mobilized	11	23.3	12.3	3.1	No
	12	14.6	10.6	21.5	Yes
	13	11.3	4	1.5	No
	14	24.7	15.3	14.5	Yes
	15	10.2	27.7	46.6	Yes
	16	23.6	62.1	51	Yes
	17	28.9	55.4	67.5	Yes
	18	26.9	33.5	32	Yes
	19	32.8	56.9	32.8	Yes
AM1421-mobilized	21	6.7	17.7	1.4	No
	22	9.2	12.5	4.5	Yes
	23	6.8	4.4	22.3	Yes
	24	14.1	16.1	4.3	No
	25	10.8	14	9.1	Yes
	26	14.9	38.5	59.2	Yes
	27	36.7	50.9	63.8	Yes
	28	8.7	20	30.3	Yes
	29	9.1	40.2	46.6	yes

Mice were transplanted with either vehicle control-, AM1241-, or G-CSF–mobilized cells and presence of mobillized cells in lethally irradiated mice was determined by Ly5.2-APC staining at the indicated times. Contribution of the mobilized cells into the distinct lineages was determined by B220-APC-Cy7, CD4/CD8-FITC, and Gr1/Mac1-PE labeling of peripheral blood 20 weeks after transplantation. The numbers indicate percentages of Ly5.2 positive cells in each individual recipient mice.