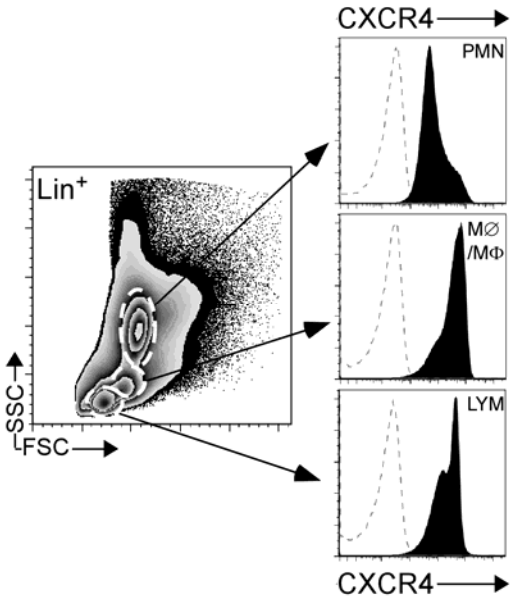
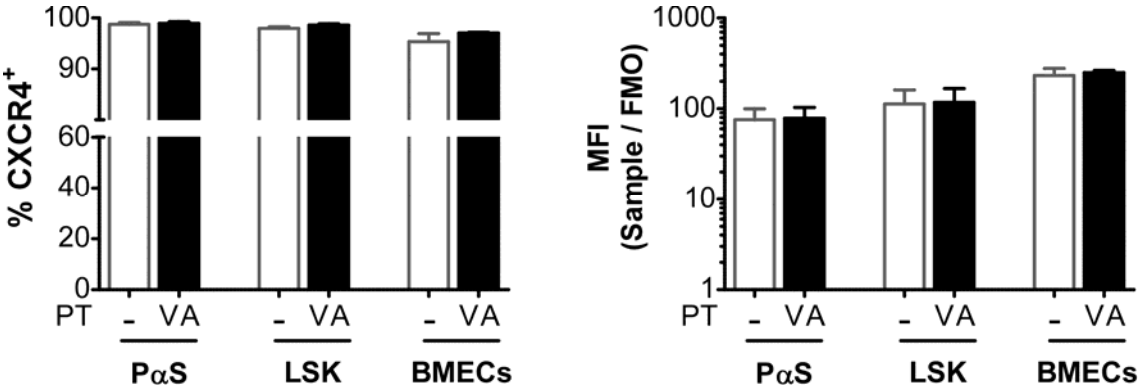
Supplementary information

Two distinct CXCR4 antagonists mobilise progenitor cells in mice by different mechanisms

Andia N. Redpath, Moïra François, Suet-Ping Wong, Dominique Bonnet, and Sara M. Rankin.

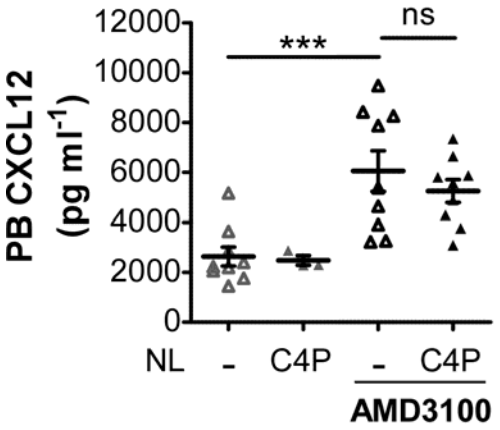


Supplemental Figure 1.CXCR4 surface expression on bone marrow leukocytes. Bone marrow cells were collected from mice by bone marrow flush for analysis of CXCR4 expression by flow cytometry. Cells were stained with antibodies to detect lineage markers. Histograms of surface CXCR4 expression on cells (shaded) as determined by flow cytometry. Open dashed-line histograms represent fluorescence minus one (FMO) controls. Data representative of 2 independent experiments. PMN, polymorphonuclear leukocytes; MØ/MΦ, monocyte/macrophages; LYM, lymphocytes.

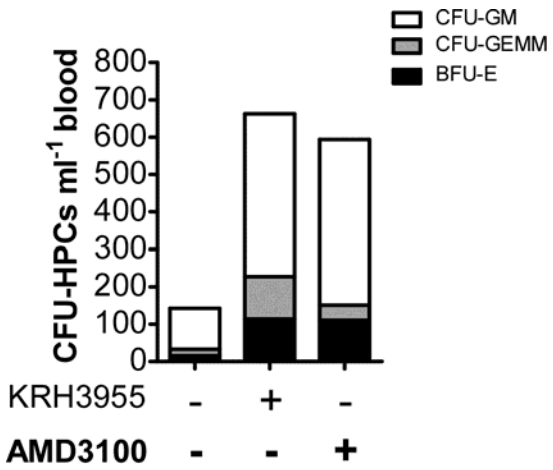


Supplemental Figure 2.VEGF treatment does not affect CXCR4 expression on bone marrow populations of interest. Mice were treated with VEGF-A (VA; 100µg.kg^{-1} mouse i.p.) or vehicle (-) once daily on 4 consecutive days. 24 hours after the last injection, mice were sacrificed. Bone marrow cells were collected by bone marrow flush for analysis of CXCR4 expression by flow cytometry. Cells were strained with antibodies to detect mesenchymal progenitor cells (PαS; TER119-CD45-CD31-PDGFRα+SCA1+), hematopoietic progenitor cells (LSK; Lin-cKit+SCA1+) and endothelial cells (BMECs; Lin-CD45-CD31+). CXCR4 positive percentage of cells within each population and calculated

mean fluorescence intensity (MFI) for CXCR4; n = 4 mice per group. Data of 2 independent experiments represented as mean ± SEM.*Control groups displayed in Figure 1.



Supplemental Figure 3.Chalcone 4-phosphate does not affect AMD3100-stimulated translocation of CXCL12 into blood. Mice were administered AMD3100 or vehicle (-) in the presence or absence of the CXCL12 neutraligand chalcone 4-phosphate (C4P; 1.5 μmol.kg^{-1} mouse i.v.), and 1 hour later blood was collected for quantification of CXCL12 in PB plasma; *n* = 3-9 mice per group. CXCL12 levels are shown as pg.ml^{-1} Data of at least 2 independent experiments (except C4P alone) represented as mean ± SEM; ***P< 0.001, ns, not significant (one-way ANOVA with Bonferroni test).



Supplemental Figure 4. Mobilisation of HPCs by distinct CXCR4 antagonists. Mice were administered KRH3955, AMD3100 or vehicle (-), 2 hours and 1 hour prior to the cull, respectively. Blood was collected for analysis of circulating CFU-HPCs; n = 4-8 mice per group. CFU-HPCs are shown as colonies per ml blood. BFU-E, erythroid progenitors (burst-forming unit–erythrocyte); CFU-GEMM, multipotential progenitor cells (colony-forming unit–granulocyte, erythrocyte, macrophage, megakaryocyte); CFU-GM, granulocytic–

monocytic progenitors (colony-forming unit-granulocyte, macrophage). *n* = 5-6 mice per group. Data of 2 independent experiments represented as mean ± SEM.

Supplemental Methods

Antibody	Clone	Company
CD45 - PB	30-F11	Invitrogen
CD31 - PB	390	Invitrogen
CD31 - FITC	390	Biolegend
PDGFRα - APC	APA5	eBioscience
SCA-1 - PeCy7	D7	BD Biosciences
Lin - PB	17A2/RB6-8C5/RA3-6B2/Ter-119/M1/70	Biolegend
c-Kit - FITC	2B8	Biolegend
TER119 – V450	TER-119	BD Biosciences
CXCR4 - PE	L276F12	Biolegend

Supplemental Table 1. List of antibodies

	No. mice per group					
Figure	**A**	**B**	**C**	**D**	**E**	**F**
1A-C	4	4	4	-	-	-
2A-B	14	11	11	11	-	-
2C	12	8	8	7	-	-
2D	14	14	11	10	-	-
2E-F	10	10	5	7	-	-
3A	12	7	8	9	-	-
3B	15	14	6	14	-	-
3C	10	8	6	6	9	8
4A-B	10	10	8	7	-	-
4C	15	17	8	7	-	-
4D	14	16	8	7	-	-
4E-F	8	4	8	7	-	-
5A-B	6	6	5	8	-	-
5C	5	5	5	7	-	-
6A	5	5	7	-	-	-
6B	5	6	7	-	-	-

Supplemental Table 2. Number of mice per group displayed in main figures.