Supplemental data for

Bone marrow cell trafficking analyzed by ^{89}Zr-oxine positron emission tomography in a murine transplantation model

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Supplemental Materials and Methods

Evaluation of free ^{89}Zr accumulation in the bone and prevention by deferoxamine

Free ^{89}Zr can be released from the cells died after the transfer and could be taken up by the hydroxyapatite of the bone (37), causing ambiguity of the ^{89}Zr signal (labeled cells homed to the BM vs free ^{89}Zr). To evaluate the extent of free ^{89}Zr accumulation in the bone, we used deferoxamine (DFO) to capture the free ^{89}Zr, which is excreted from the kidney. We then compared the whole-body and bone/BM radioactivities with those of mice without DFO treatment. Two groups of mice received injections of ^{89}Zr-oxine labeled BM cells ($2.1x10^7$ cells at 8.8 kBq/10^6 cells), one of which received DFO (660 μg) intramuscularly 15 min before and 1, 2, 3 and 4 h after cell transfer and twice daily for 7 days thereafter. MicroPET/CT imager was used to acquire images and activities in the whole-body and the

BM/bone were quantified using VivoQuant software.

Evaluation of side effect by ^{89}Zr retention in the spine

^{89}Zr in 1 M oxalic acid (74 MBq) was neutralized using 2 M Na_2CO_3, and added 0.5 M 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) and water to adjust to 1 ml, 0.15 M HEPES. To evaluate for potential toxicity induced by retention of ^{89}Zr in the BM or bone, 92.5 kBq of neutralized ^{89}Zr solution diluted in PBS was injected via the tail vein to have the ^{89}Zr taken up by the bone (n=3). A group of mice also received DFO to clear the ^{89}Zr from the kidney, preventing the uptake to the bone (n=3). The control group remained untreated (n=3).

Supplemental Figures

Figure S1

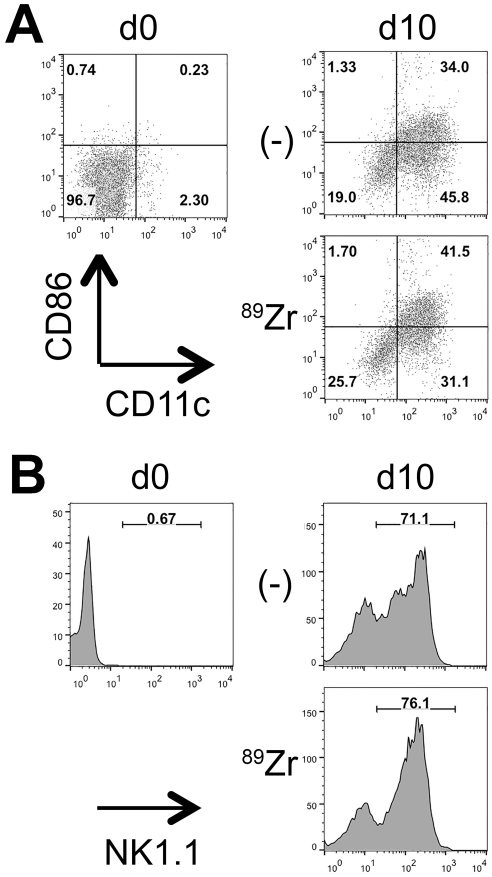

Figure S1. ^{89}Zr-labeled BM cells differentaiate into DCs and NK/NK-T cells.

^{89}Zr-oxine labeled BM cells (22.2 kBq/10^6 cells) cultured with 20 ng/ml GM-CSF (A) or 25 nM of IL-15 (B) differentiated into mature DCs (A) and NK/NK-T cells (B), respectivly, after a 10 day-culture, in a comparable manner to the non-labeled cells (representative data of 4 independent experiments).

Figure S2

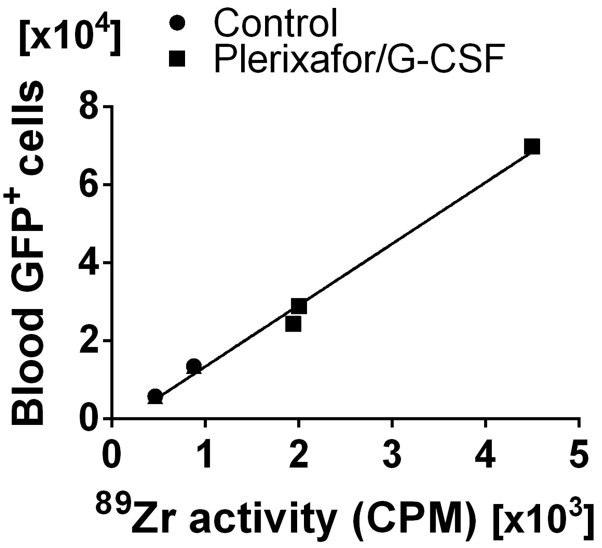

Figure S2. ^{89}Zr activity of blood correlate well with the number of ^{89}Zr-labeled donor cells in the circulation.

Twenty million GFP$^+$ BM cells labeled with ^{89}Zr-oxine (3.7-11.1 kBq/10^6 cells) were transplanted to two groups of mice, one of which received plerixafor/G-CSF injection at 3 h and 1 d. Blood was collected from the mice 2 h after the second mobilization treatment and span to isolate cells. ^{89}Zr activity of cells in the blood counted by the γ-counter correlated well with GFP$^+$ cell number counted by flow cytometry analysis of the same sample. Pearson correlation coefficient (r) was 0.9912.

Figure S3

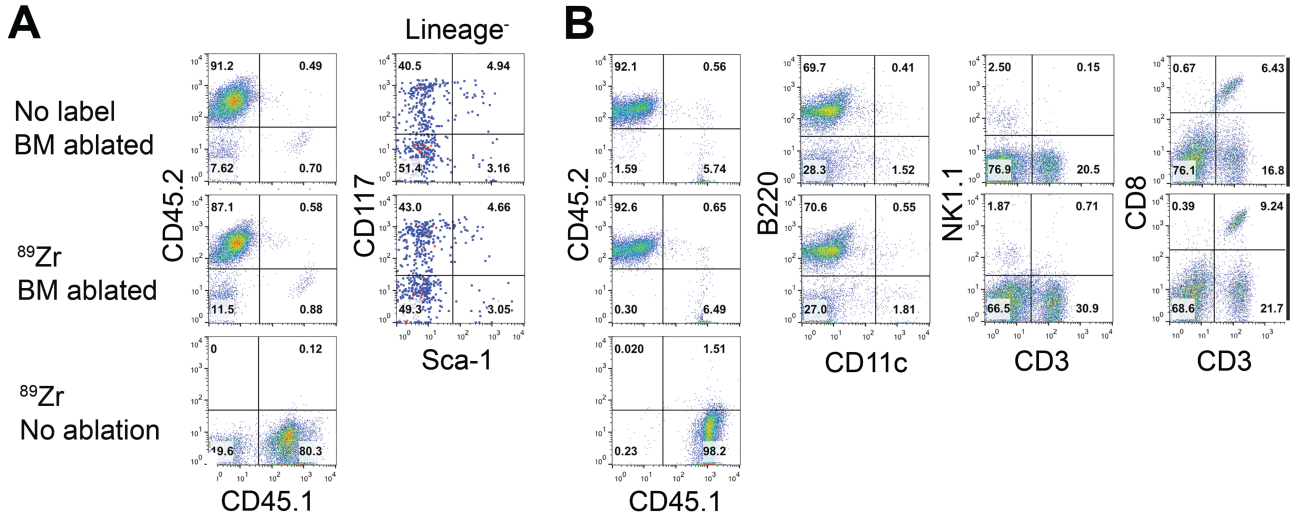

Figure S3. Donor BM cells labeled with ^{89}Zr-oxine engraft and differentiate into mature immune cells in the recipient mice.

Ten weeks following transfer of non-labeled or ^{89}Zr-labeled BM cells to mice with or without BM ablation, BM and splenocytes of the recipient were analyzed by flow cytometry. A. CD45.2$^+$ donor derived cells reconstituted the BM of the BM-ablated hosts (CD45.1$^+$), which did not occur in the non-BM ablated hosts. In BM-ablated hosts, ^{89}Zr-labeling of donor cells did not affect the engraftment of hematopoietic stem/progenitor cells (CD45.2$^+$lineage marker$^-$sca-1$^+$CD117$^+$ cells) (Representative data of 3 experiments).

B. Analysis of splenocytes revealed that CD45.2$^+$ donor BM cells differentiated to mature immune cells such as DCs (CD11c$^+$), B cells (B220$^+$), NK cells (CD3$^-$NK1.1$^+$), and T cells (CD3$^+$NK1.1$^-$) in BM-ablated hosts but not in non-ablated recipient mice (Representative data of 3 experiments).

Figure S4

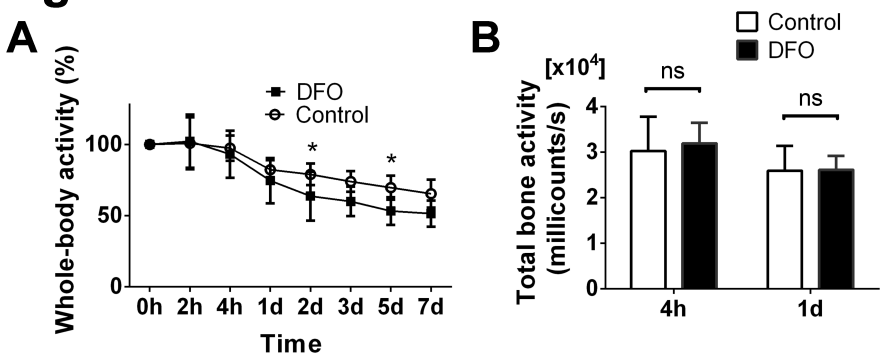

Figure S4. Accumulation of free ^{89}Zr is observed from 2 days after the BM cell transfer.

Mice (n=3) were administered with deferoxamine (DFO) chelator intramuscularly at a dose of 660 μg 15 min prior to and at every hour for the first 4 hours after ^{89}Zr-oxine labeled BM cell transfer ($2x10^7$ cells at 8.8 kBq/10^6 cells) and twice daily thereafter up to day 7. Serial microPET/CT images were acquired and ^{89}Zr activity in the whole-body and bone/BM was quantified. A. No significant enhancement of ^{89}Zr clearance by DFO (square) was observed up to 1 d. After 2 d, DFO induced excretion of approximately 15% of ^{89}Zr from the whole-body compared to control (circle), suggesting that no significant free ^{89}Zr was retained in the body even without the use of DFO until day 1. Each asterisk indicates statistical significance (*:$P < 0.05$, Two-way analysis of variance followed by Sidak's multiple comparisons test correction). B. ^{89}Zr activity in the bone/BM was similar between the two groups at 4 h and 1 d (both $P = 0.8192$, Unpaired two-tailed t-test).

Figure S5

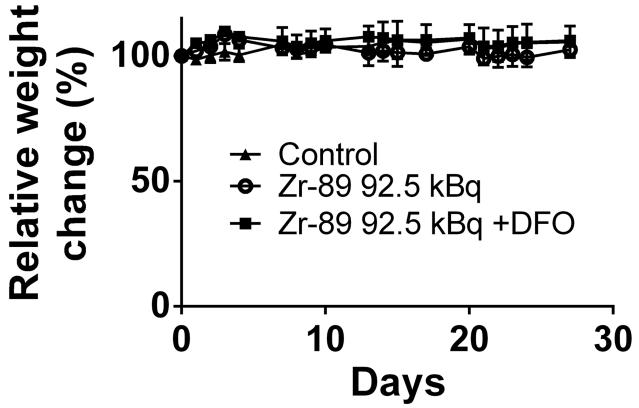

Figure S5. **^{89}Zr retention in the vertebra does not induce body weight loss**

Mice received injections of a high dose of free ^{89}Zr (92.5 kBq) alone or in combination with DFO (n=3). Body weights were recorded for 27 days. No significant body-weight loss was observed in the experimental groups compared to the control (P = 0.3651, One-way analysis of variance followed by the Brown-Forsythe test correction).