Supplementary Materials and Methods

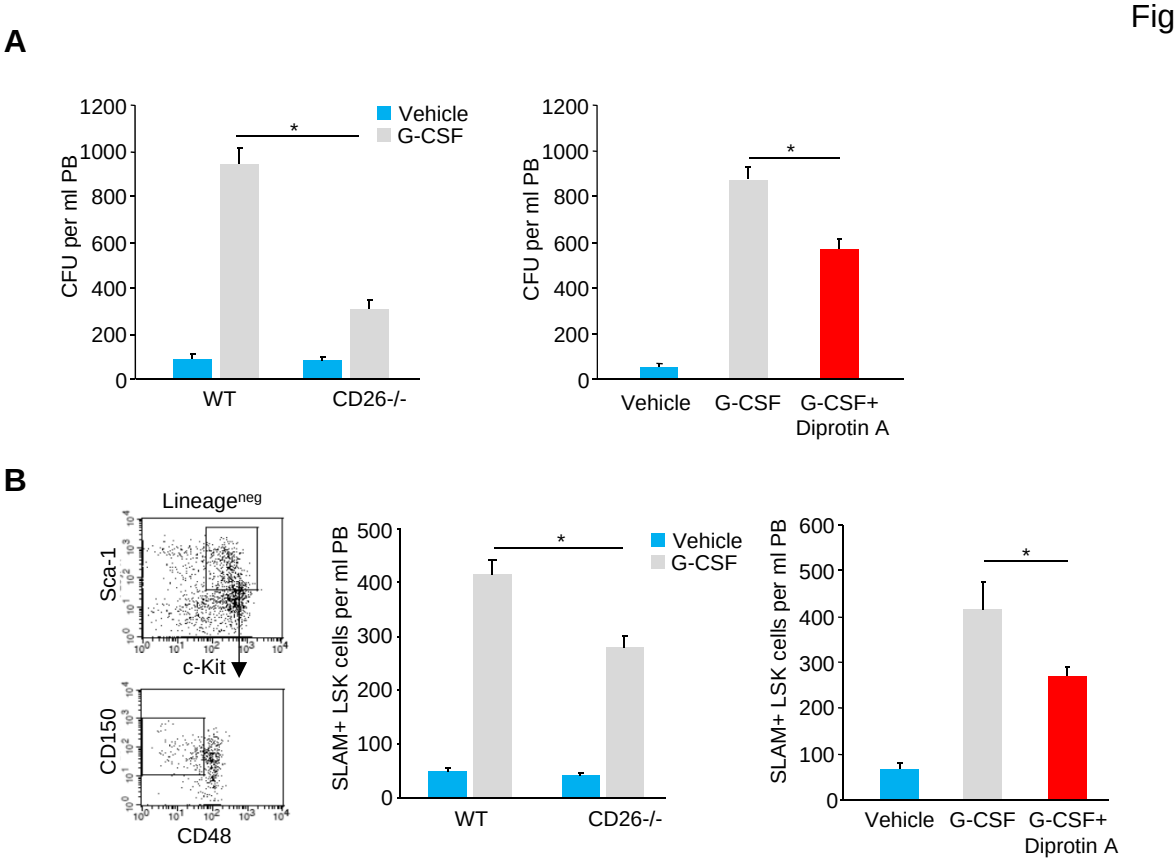
QRT-PCR

Mouse bone marrow endothelial cells were treated by G-CSF, G-CSF plus diprotin A, G-CSF plus diprotin A with NPY or G-CSF plus diprotin A with NPY_{3-36}. Total RNA isolated from above treated cells was used for quantitative RT-PCR using Power SYBR Green PCR mix (Applied Biosystems Inc, Foster City, CA, USA). Primers for the RT-PCR experiments are as previous described (1). Difference in mRNA were calculated using the ΔCT method and were normalized to GAPDH.

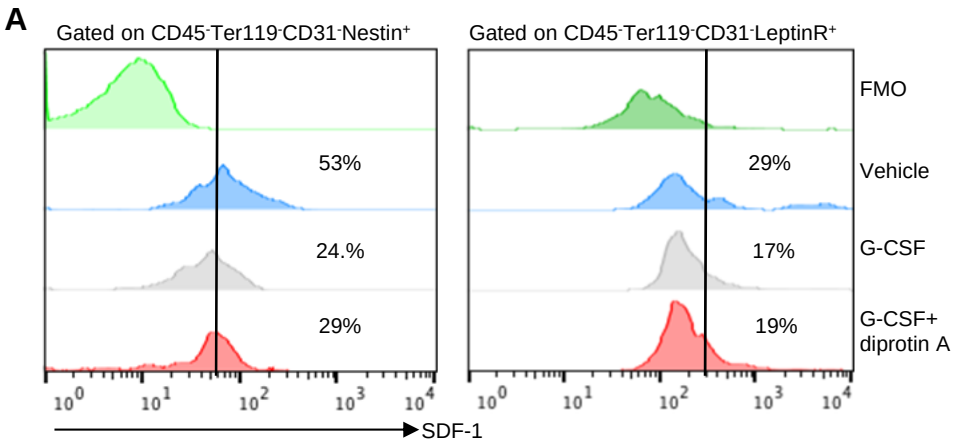
Reference List

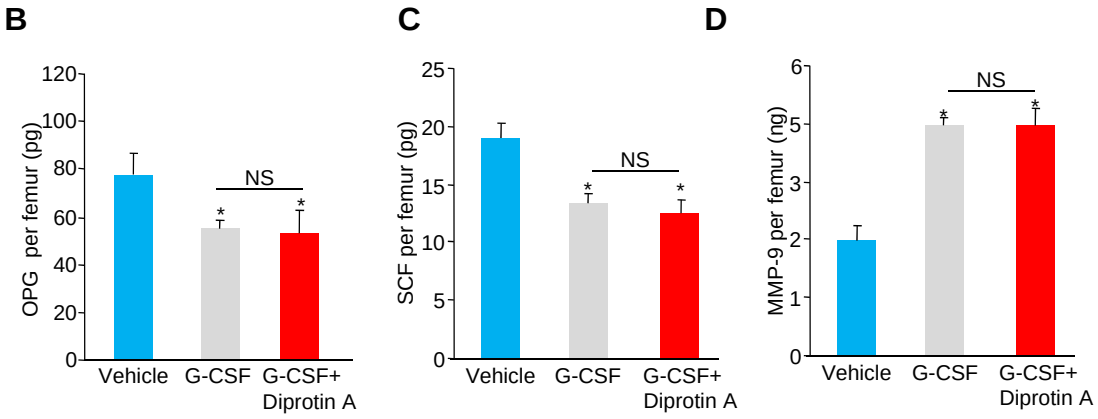
1. Li,J., Qu,X., Yao,J., Caruana,G., Ricardo,S.D., Yamamoto,Y., Yamamoto,H., and Bertram,J.F. 2010. Blockade of endothelial-mesenchymal transition by a Smad3 inhibitor delays the early development of streptozotocin-induced diabetic nephropathy. *Diabetes* 59:2612-2624.

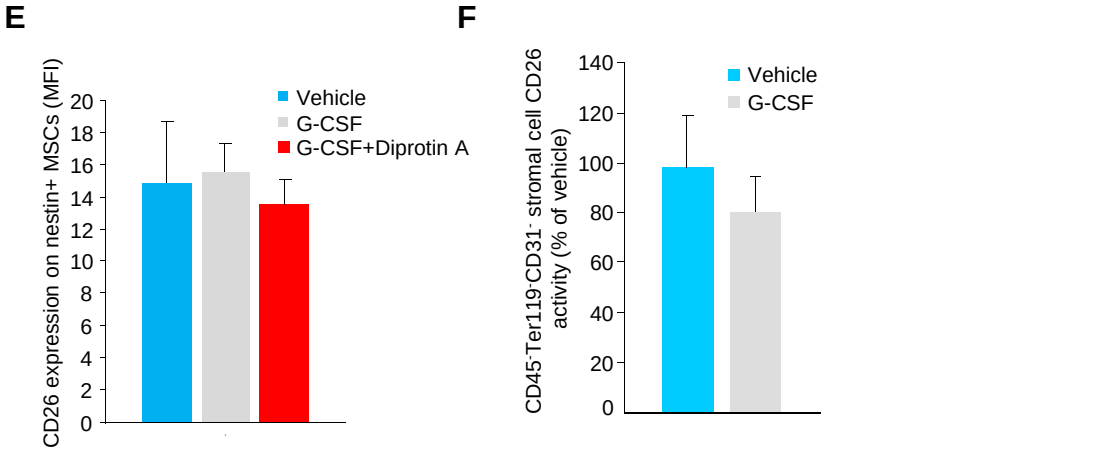
Supplemental Figure 1



Supplemental
Figure 2

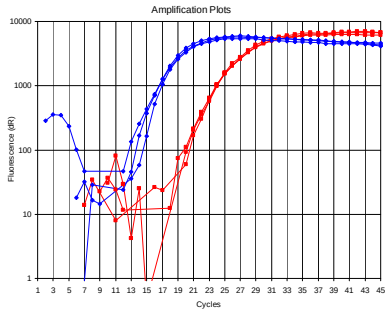


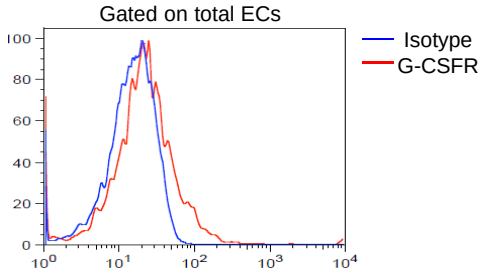




Supplemental
Figure 3

A





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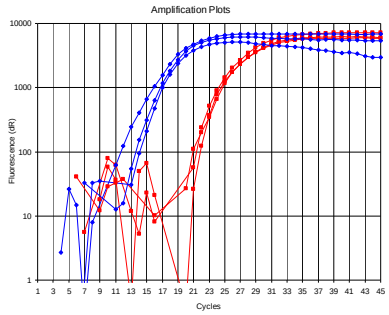
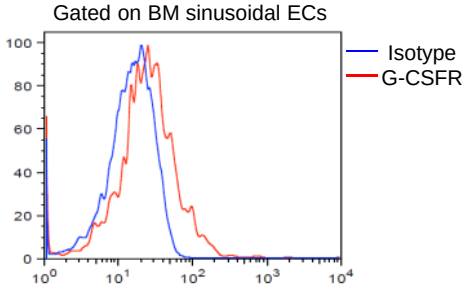
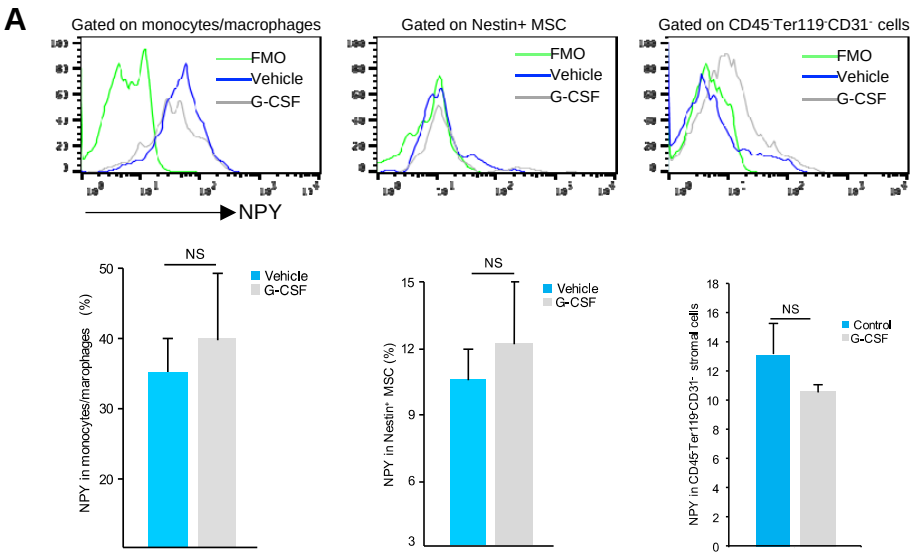
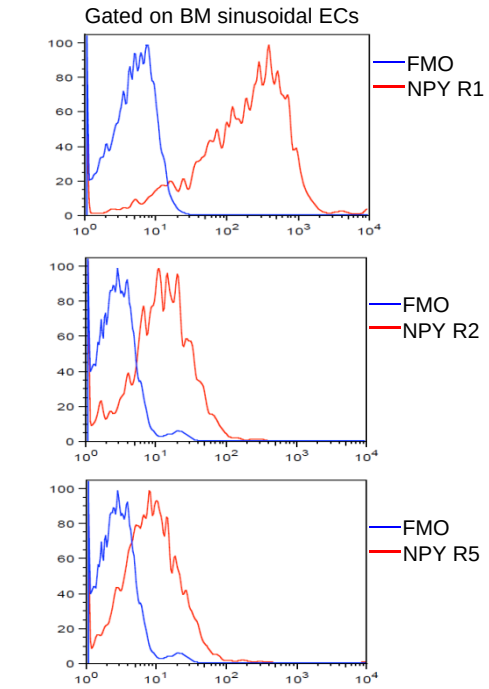


HPRT
G-CSFR

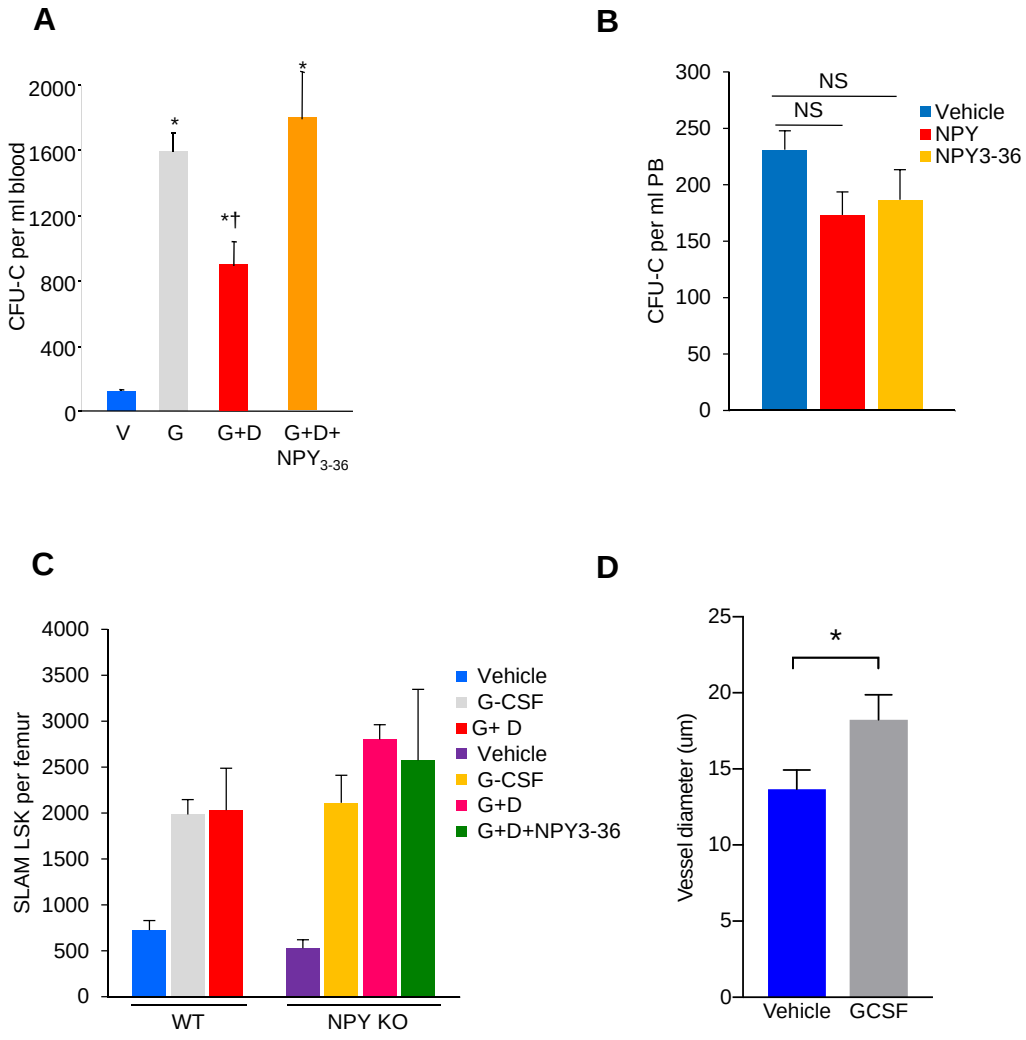


Supplemental
Figure 4

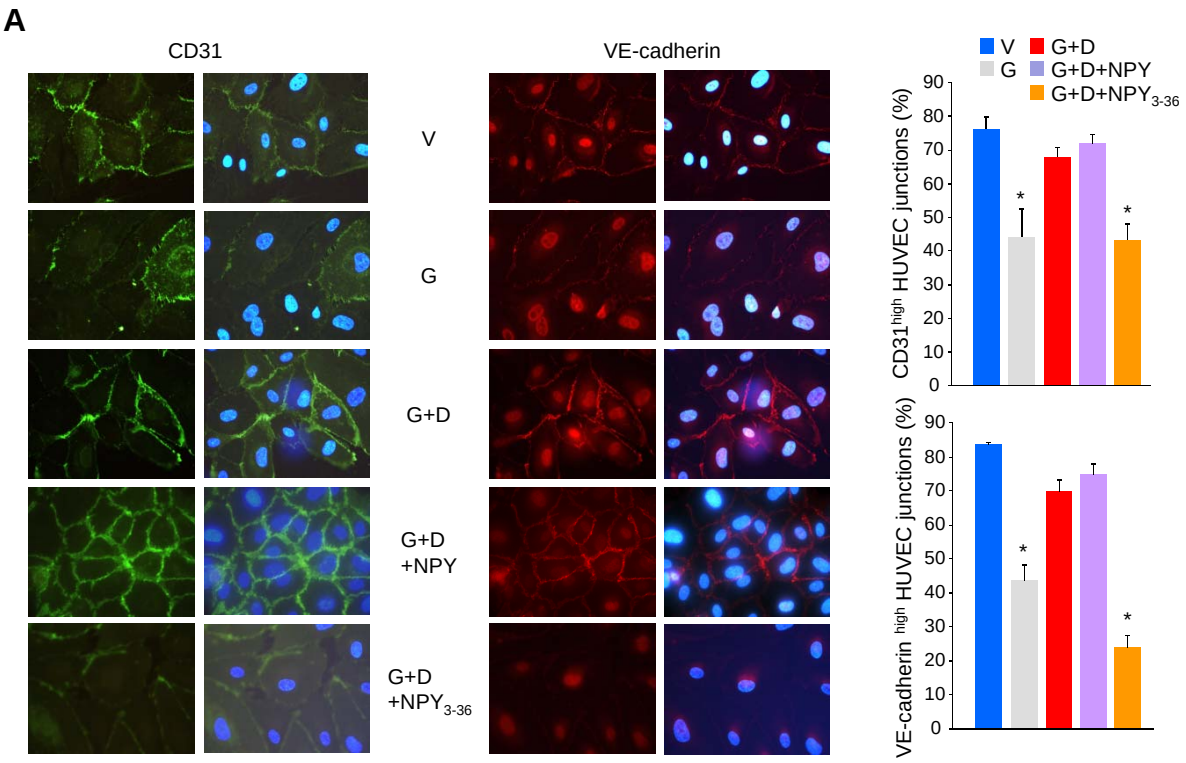


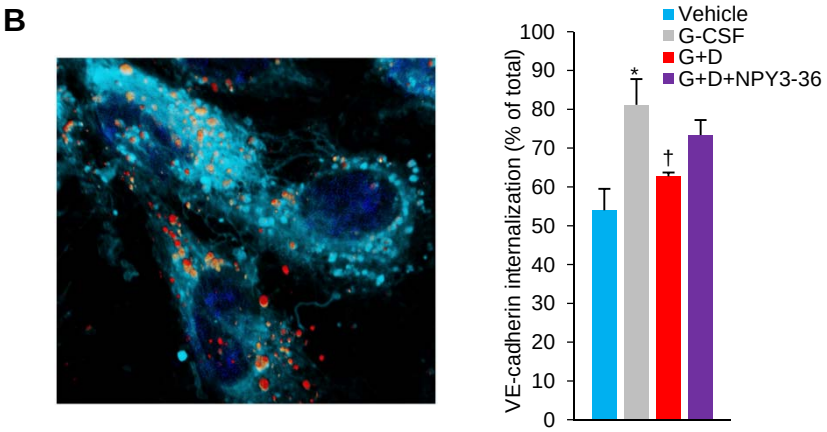


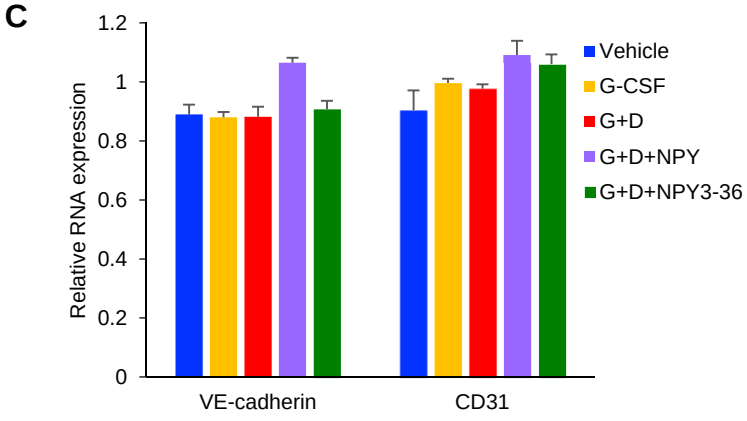
Supplemental
Figure 5



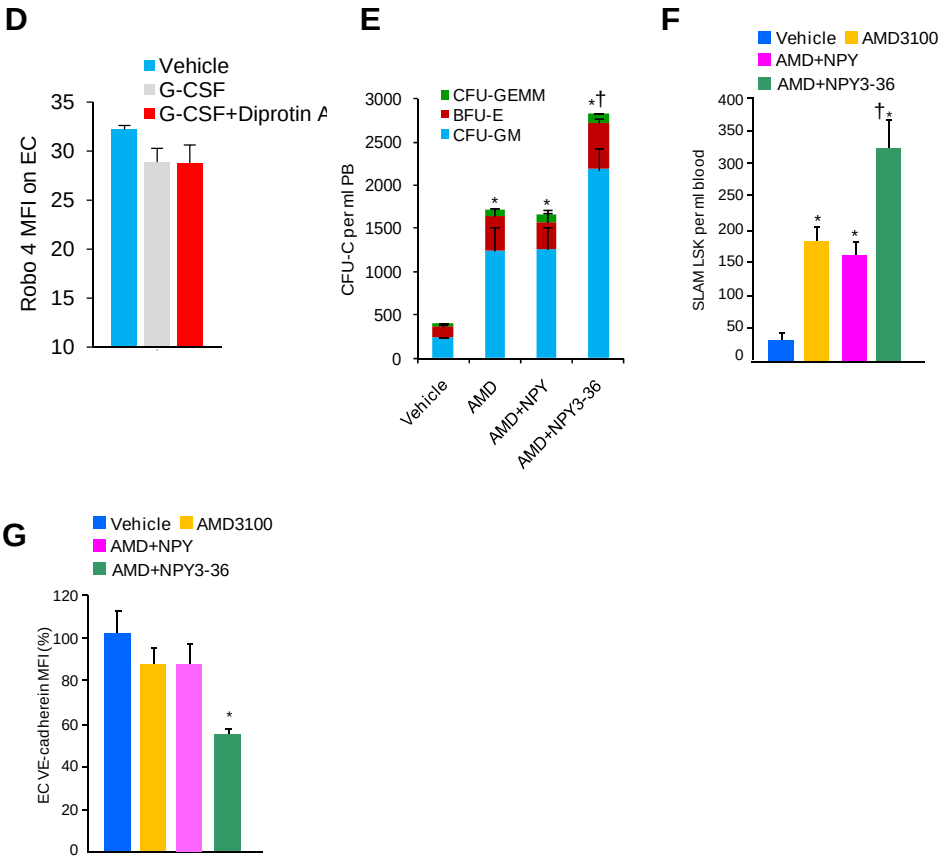
Supplemental
Figure 6







Supplemental Figure 6



Supplementary Figure Legends

Figure S1. HSPC mobilization and CD26 expression measurement

(A) CFU and (B) SLAM LSK cell numbers in PB of G-CSF mobilized wild-type control mice, $CD26^{-/-}$ mice and diprotin A treated mice (X±SEM; N= 5 mice/group, each assayed individually). (C) Competitive transplantation using equal volumes of PB from G-CSF or G-CSF plus diprotin A (CD45.2 C57BL/6) treated mice in combination with 2×10^5 BM competitor cells from CD45.1 BoyJ mice. PB donor chimerism was measured by flow cytometry at 6 months after transplantation (X±SEM; N= 5 mice/group, each assayed individually). (D) Flow cytometry analysis of CD26 expression on BM LSK and SLAM LSK of mice treated with G-CSF (X±SEM; N= 2 independent experiments, N=4 mice/group). $p<0.05$ compared to G-CSF treated WT mice using 1-way ANOVA with Sidak's multiple comparisons test (A-C) or Student T-test (D).

Figure S2. Hematopoietic stem cell retention factors and CD26 expression/activity in BMEF of G-CSF and G-CSF plus diprotin A treated mice

(A) Representative flow cytometry plots showing intracellular SDF-1 expression in mouse BM nestin+ MSCs (left) and leptin receptor+ mesenchymal stromal cells (right). (B, C & D) Mice were treated with G-CSF alone or with diprotin A and OPG, SCF and MMP-9 levels in BMEF were measured by ELISA (X±SEM of at least four mice/group each assayed individually). (E) Flow cytometry analysis of CD26 expression on BM $nestin^+$ MSCs of vehicle, G-CSF and G-CSF plus diprotin A treated mice (X±SEM; N= 4 mice/group). (F) Effect of G-CSF treatment on stromal cells ($CD45^-Ter119^-CD31^-$) DPP IV activity (X±SEM; N= 4 mice/group). $p<0.05$ compared to vehicle using 1-way ANOVA with Sidak's multiple comparisons test (B-E) or Student T-test (F).

Figure S3. G-CSF receptor expression on mouse BM endothelial cells

G-CSF receptor mRNA and protein expression on (A) BM total ECs and (B) sinusoidal ECs. BM total EC and sinusoidal ECs were sorted by FACS. G-CSF receptor mRNA expression in these cell populations were quantitated by QRT-PCR. G-CSF receptor protein expression on the cell surface of BM ECs was determined by flow cytometry (X±SEM; N= 4 mice/group).

Figure S4. NPY and NPY receptors expression on bone marrow cells

Intracellular NPY expression in (A) bone marrow Gr-1$^-$M-CSFR$^+$F4/80^{int} monocytes/macrophages, nestin$^+$ MSCs, and CD45$^-$Ter119$^-$CD31$^-$ stromal cells in response to G-CSF treatment (X±SEM of ≥ 4 mice/group). (B) Representative flow cytometry plots for NPY1, NPY2 and NPY5 receptors expression on mouse BM sinusoidal ECs. $p<0.05$ compared to vehicle using Student T-test.

Figure S5. Effect of cleaved NPY on HSPCs trafficking

(A) HPC mobilization in WT mice treated with G-CSF alone, G-CSF plus diprotin A and G-CSF, diprotin A plus NPY_{3-36}. HPC mobilization was determined by CFU-C assay (X±SEM; N= 4 mice/group, each assayed individually). (B) HPC mobilization in response to NPY or NPY_{3-36} treatment (X±SEM; N= 4 mice/group, assayed individually). (C) SLAM LSK in BM of WT and $NPY^{-/-}$ mice treated with G-CSF, G-CSF plus diprotin A, and G-CSF plus diprotin A with NPY_{3-36} (X±SEM; N= 4 mice/group, assayed individually). (D) Intravital 2-photon image analysis of the vessels diameter in isolectin B4 labeled evaluable vessels in BM from control and G-CSF treated mice (X±SEM; N= 4 mice/group, minimum 15 vessels). * $p<0.05$ compared to vehicle and † $p<0.05$ compare to G-CSF using 1-way ANOVA with Sidak's multiple comparisons test (A&B) or Student T-test (D).

Figure S6. Endothelial cell junction proteins expression and HSPC mobilization in response to AMD3100

(A) Left: Representative immunofluorescence images showing CD31 expression on cell junctions of HUVEC without or with dapi staining. HUVEC were treated with G-CSF, G-CSF plus diprotin A, G-CSF plus diprotin A with NPY or with NPY_{3-36} (magnification 200X). Middle: VE-cadherin expression on cell junctions of HUVEC without or with dapi staining. Cells were treated as described above (magnification 200X). Right: Bar graphs showing average values for CD31 (upper), and VE-cadherin (lower) at HUVEC junctions (images were evaluated in triplicate samples for 5 areas/ sample). (B) Left: Representative confocal image of HUVEC showing total (red) and intracellular (cytoplasmic) VE-cadherin expression (MFI) (scale bar: 10 um) . Right: Average of internalized VE-cadherin in HUVEC after G-CSF or G-CSF plus diprotin A or G-CSF plus diprotin A with NPY_{3-36} treatment (X±SEM; N= minimum 3 focus area/treatment). (C) Expression of VE-cadherin and CD31 transcripts in EC in response to G-CSF, G-CSF plus diprotin and G-CSF plus diprotin A with NPY or NPY_{3-36} (X±SEM; N=3 experiment/group). (D) Robo4 expression on BM EC from mice treated with G-CSF or G-CSF plus diprotin A (X±SEM; N=4 mice/group). (E&F) CFU-Cs and SLAM LSK mobilization in response to AMD3100 or AMD3100 plus NPY or AMD3100 plus NPY_{3-36} (X±SEM; N= 4 mice/group, each assayed individually). (G) VE-cadherin expression on BM SECs of mice treated with AMD3100 or AMD3100 plus NPY or AMD3100 plus NPY_{3-36} (X±SEM; N= 4 mice/group, each assayed individually). * $p<0.05$ compared to vehicle and † $p<0.05$ compare to G-CSF/AMD3100 using 1-way ANOVA with Sidak's multiple comparisons test.