SUPPLEMENTARY APPENDIX

Bone marrow adipose tissue-derived stem cell factor mediates metabolic regulation of hematopoiesis

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 doi:10.3324/haematol.2018.205856

Received: September 3, 2018.

Accepted: February 18, 2019.

Pre-published: February 21, 2019.

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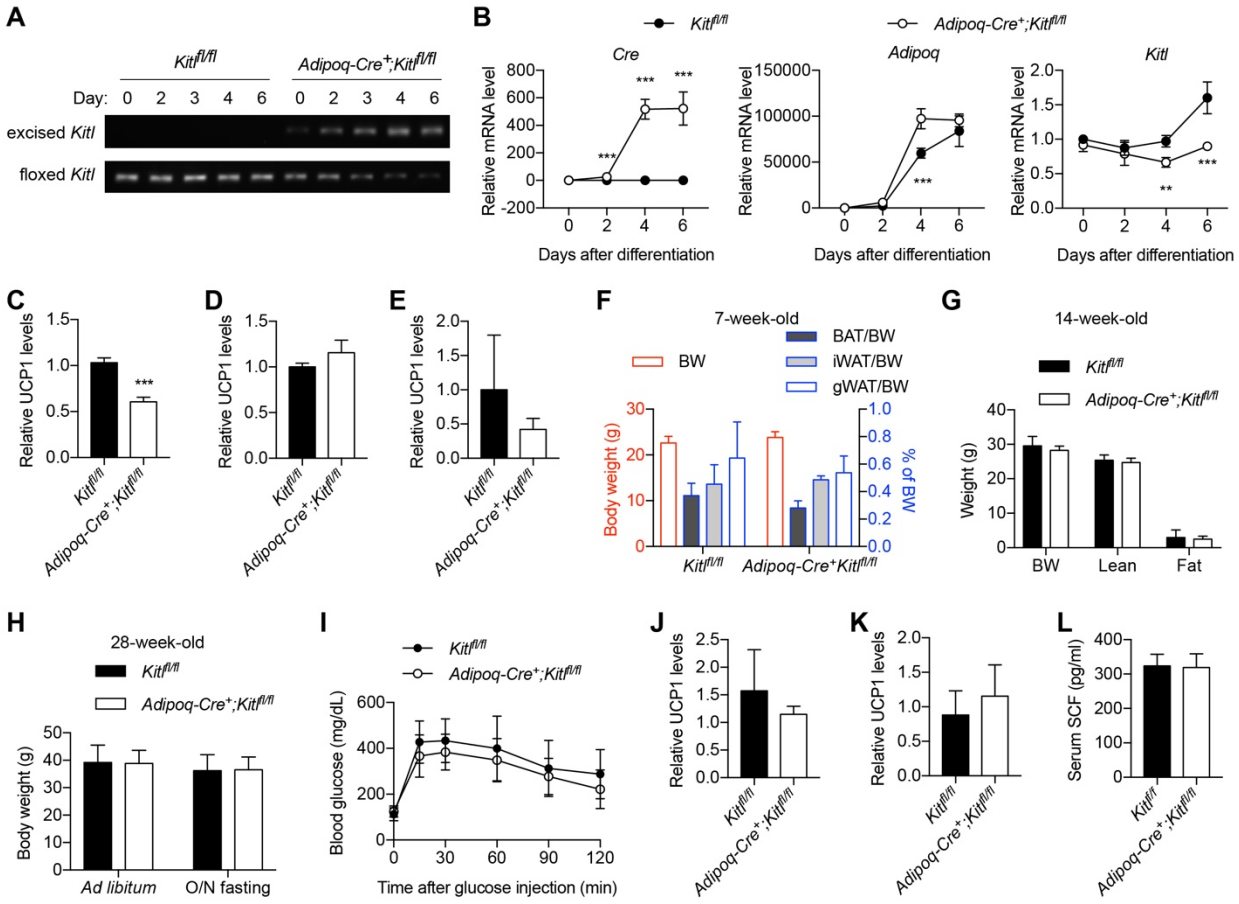

Fig. S1. Related to Fig. 1.

(A) SVF cells from iWAT of *Kitl*$^{fl/fl}$ and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ mice were induced to adipocytes in vitro. DNA was collected at indicated days for PCR analysis.

(B) SVF cells from iWAT of *Kitl*$^{fl/fl}$ and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ mice were induced to adipocytes in vitro. RNA samples at indicated days were collected for qRT-PCR.

(C-E) Densitometric analysis of UCP1 shown in Fig. 1B-D, respectively.

(F) Body weight and mass of BAT, iWAT, and gWAT of 7-week-old *Kitl*$^{fl/fl}$ (n = 3) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 4) male mice.

(G) Body composition of 14-week-old *Kitl*$^{fl/fl}$ (n = 6) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 10) male mice.

(H, I) 28-week-old *Kitl*$^{fl/fl}$ (n = 7) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 6) male mice were fasted overnight, body weight (H) and glucose tolerance test (I) were determined.

(J, K) Densitometric analysis of UCP1 in BAT (J) and iWAT (K) shown in Fig. 1F.

(L) Levels of serum SCF determined by ELISA (n = 4-5).

Data are presented as mean ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001 by unpaired student's t test.

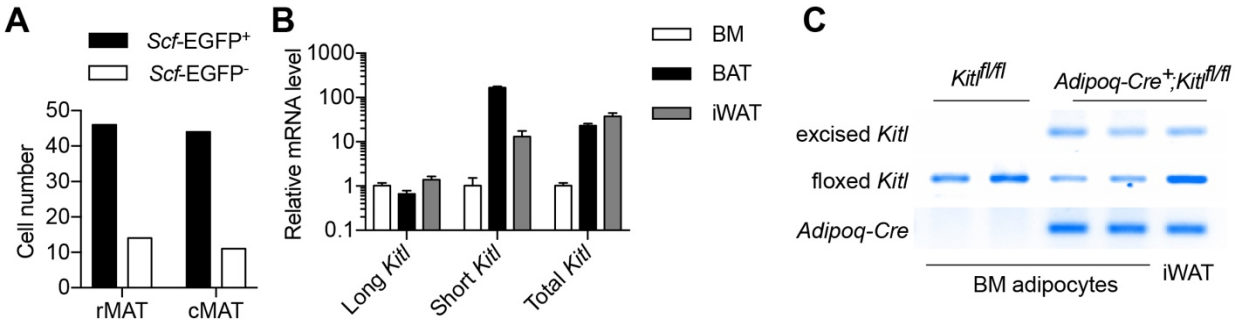

Fig. S2. Kitl expression in adipose tissues and Kitl gene deletion in *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ mice.

(A) Numbers of *Scf*-EGFP^{+} and *Scf*-EGFP^{-} BM adipocytes counted in rMAT and cMAT of *Kitl*^{EGFP} knockin mice.

(B) Relative levels of long, short, and total *Kitl* in flushed BM (n = 3-6), BAT (n = 6-7), and iWAT (n = 5).

(C) Mature adipocytes were purified from the BM of *Kitl*$^{fl/fl}$ and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ mice and then subjected to DNA extraction and PCR analysis of the *Kitl* gene.

Data are presented as mean ± SD (B).

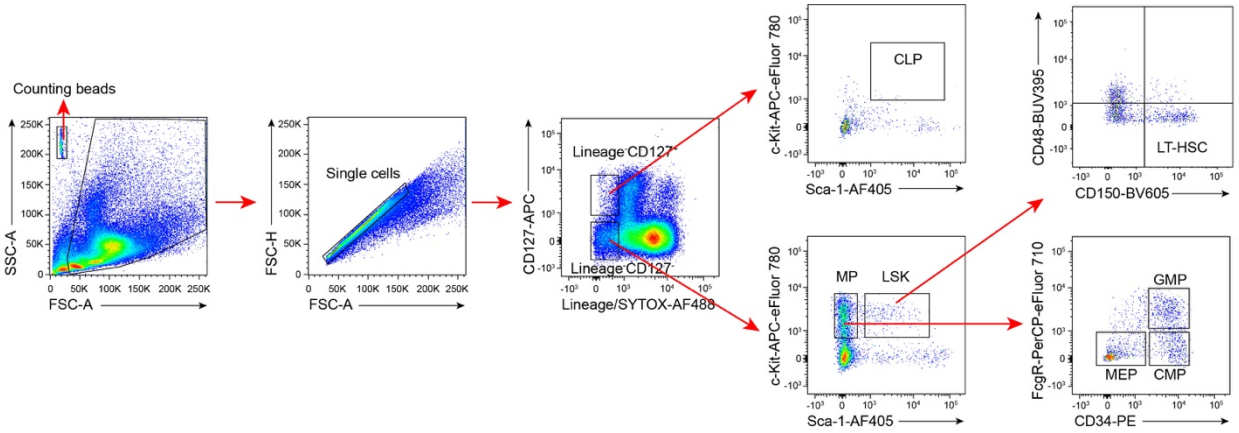

Fig. S3. Gating strategy for flow cytometry of bone marrow hematopoietic stem and progenitor cells.

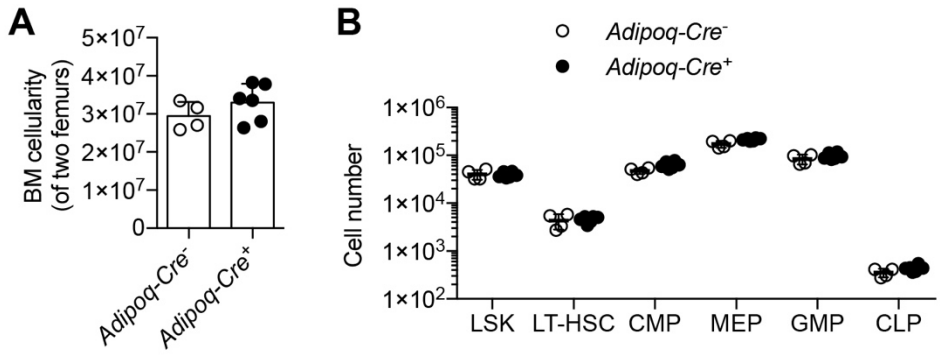

Fig. S4. No Cre-specific effect on hematopoiesis in the *Adipoq-Cre* line.

(A) Bone marrow cellularity of 4 weeks old *Adipoq-Cre*$^{-}$ (n = 4) and *Adipoq-Cre*$^{+}$ (n = 6) male mice.

(B) Quantification of HSPCs and progenitors of 4 weeks old *Adipoq-Cre*$^{-}$ (n = 4) and *Adipoq-Cre*$^{+}$ (n = 6) male mice.

Data are presented as mean ± SD.

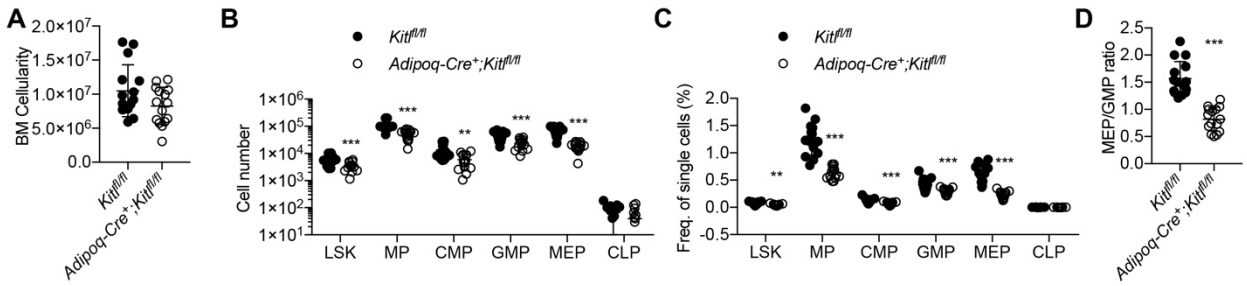

Fig. S5. Adipose-secreted SCF is required for hematopoietic stem and progenitor cells in females.

(A) BM cellularity in the femur of 14-week-old *Kitl*$^{fl/fl}$ and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ female mice (n = 15).

(B, C) Absolute numbers (B) and frequencies (C) of LSKs, MPs, CMPs, GMPs, MEPs, and CLPs in the femur of 14-week-old *Kitl*$^{fl/fl}$ and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ female mice (n = 15).

(D) The ratio of marrow MEP to GMP in 14-week-old *Kitl*$^{fl/fl}$ and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ female mice (n = 15).

Data are presented as mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by unpaired student's t test.

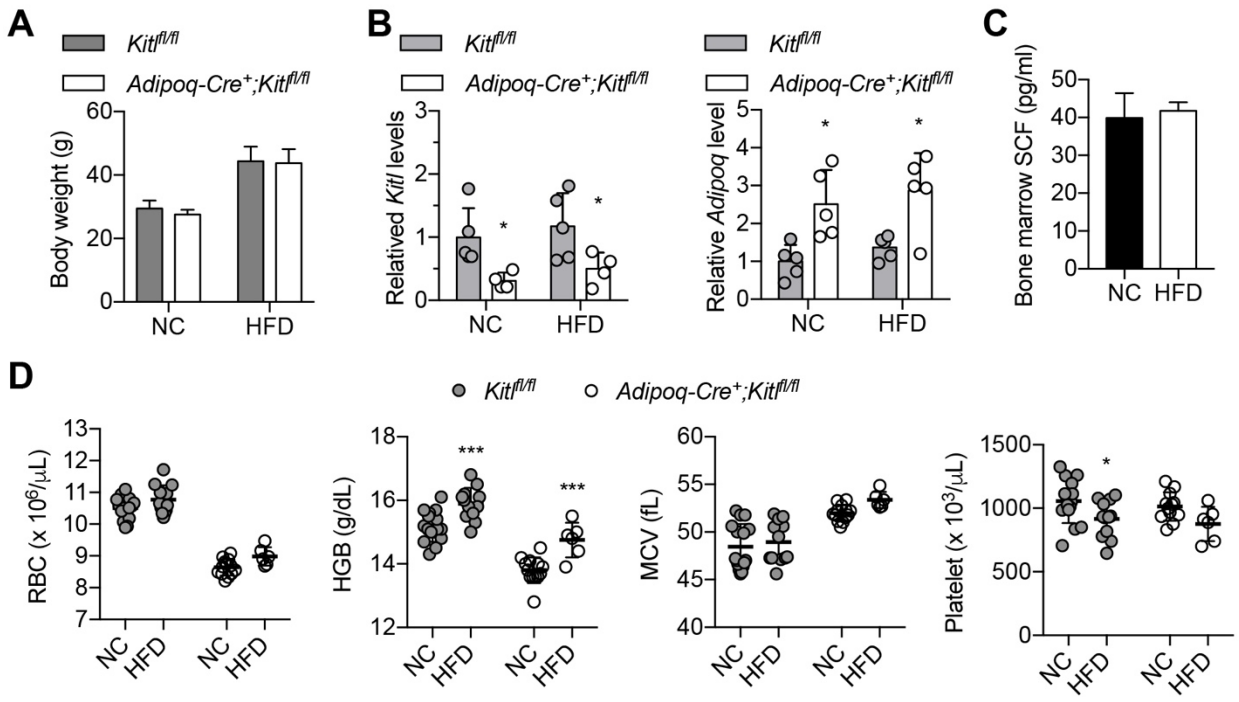

Fig. S6. Related to Figure 4.

(A) Body weight of *Kitl*$^{fl/fl}$ (n = 3 for NC and n = 6 for HFD) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 4 for NC and n = 6 for HFD) male mice fed with 8 weeks of NC or HFD.

(B) Levels of *Kitl* and *Adipoq* gene expression in the BM of *Kitl*$^{fl/fl}$ (n = 4-5) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 4-5) male mice fed with 8 weeks of NC or HFD.

(C) Levels of SCF protein in the BM supernatant from wildtype males fed with NC (n = 4) or HFD (n = 5).

(D) Complete blood count of NC- and HFD-fed *Kitl*$^{fl/fl}$ (n = 16 and 13, respectively) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 12 and 6, respectively) male mice showing RBC count, HGB, MCV, and platelet count.

Data are presented as mean ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001 Two-way ANOVA followed by multiple comparison using Sidak's correction.

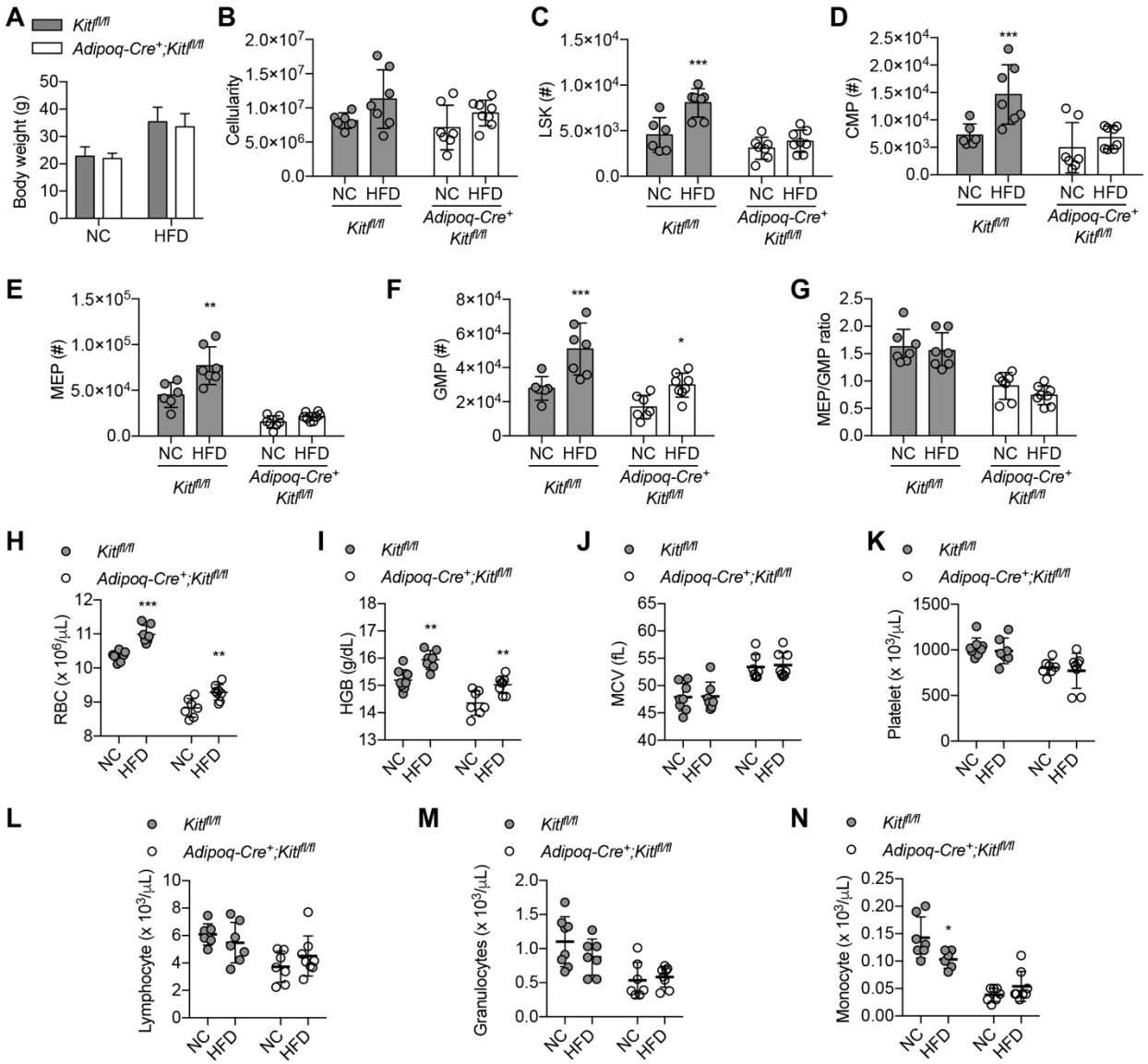

Fig. S7. HFD-stressed hematopoiesis in control and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ female mice.

8-week-old *Kitl*$^{fl/fl}$ (n = 6 for NC and n = 7 for HFD) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 7 for NC and n = 8 for HFD) female mice were fed with NC or HFD for 2 months. (A) Body weight. Bone marrow cellularity (B), numbers of LSKs, CMPs, MEPs, and GMPs (C-F) and the MEP/GMP ratio (G) were determined by flow cytometry. (H-N) Complete blood count showing RBC number (H), hemoglobin (HGB) concentration (I), MCV (J), platelet number (K), lymphocyte number (L), granulocyte number (M), monocyte number (N).

Data are presented as mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ Two-way ANOVA followed by multiple comparison using Sidak's correction.

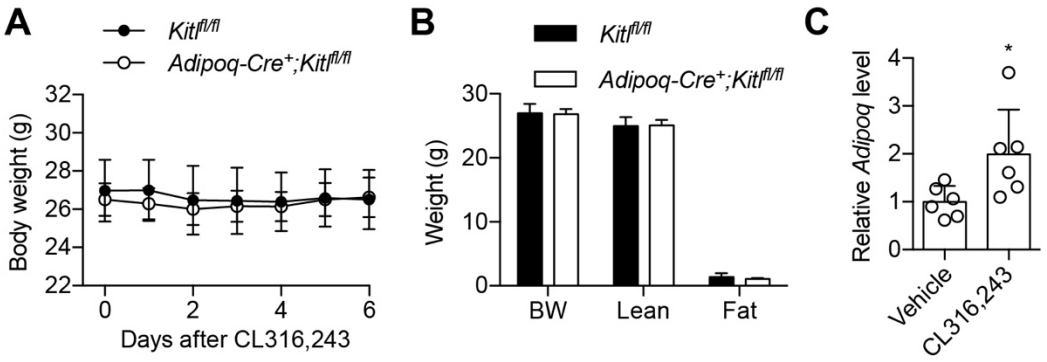

Fig. S8. Related to Fig. 5

(A) 12-week-old *Kitl*$^{fl/fl}$ (n = 7 for vehicle and n = 6 for CL) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 8 for vehicle and n = 6 for CL) male mice were treated with the saline vehicle or CL 316,243 for one week. Daily body weight (A) and body composition after 7 days of treatment (B) were determined.

(C) Relative *Adipoq* mRNA levels in the BM of wildtype mice treated with vehicle or CL 316,243 (n = 6) for one week.

Data are presented as mean ± SD. *, $P < 0.05$ by unpaired, two-tailed student's t test.

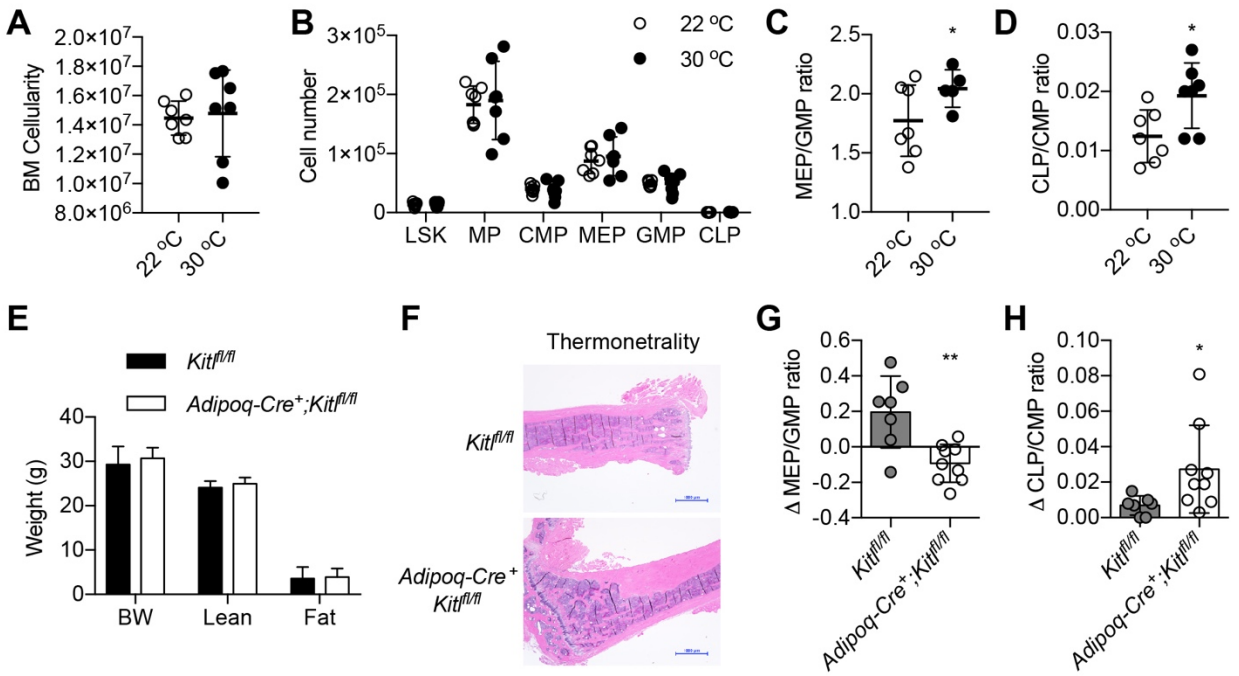

Fig. S9. Responses of HSPC to thermoneutrality.

(A-D) 13-week-old wildtype male mice were continued to be housed at 22 °C or switched to thermoneutrality for 1 month (n = 7). BM cellularity (A), HSPC numbers (B), MEP/GMP ratio (C), and CLP/CMP ratio (D) were determined by flow cytometry.

(E-H) 13-week-old *Kitl*$^{fl/fl}$ (n = 7) and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ (n = 9) male mice were housed at 30 °C for 1 month. Body composition was determined by EchoMRI (E). Representative sections of femur bone marrow (F). Changes in MEP/GMP ratio (G) and CLP/CMP ratio (H) between 22 °C and 30 °C housing were calculated for *Kitl*$^{fl/fl}$ and *Adipoq-Cre*$^{+}$*;Kitl*$^{fl/fl}$ mice.

Data are presented as mean ± SD. *, P < 0.05; **, P < 0.01 by unpaired, two-tailed student's t test.