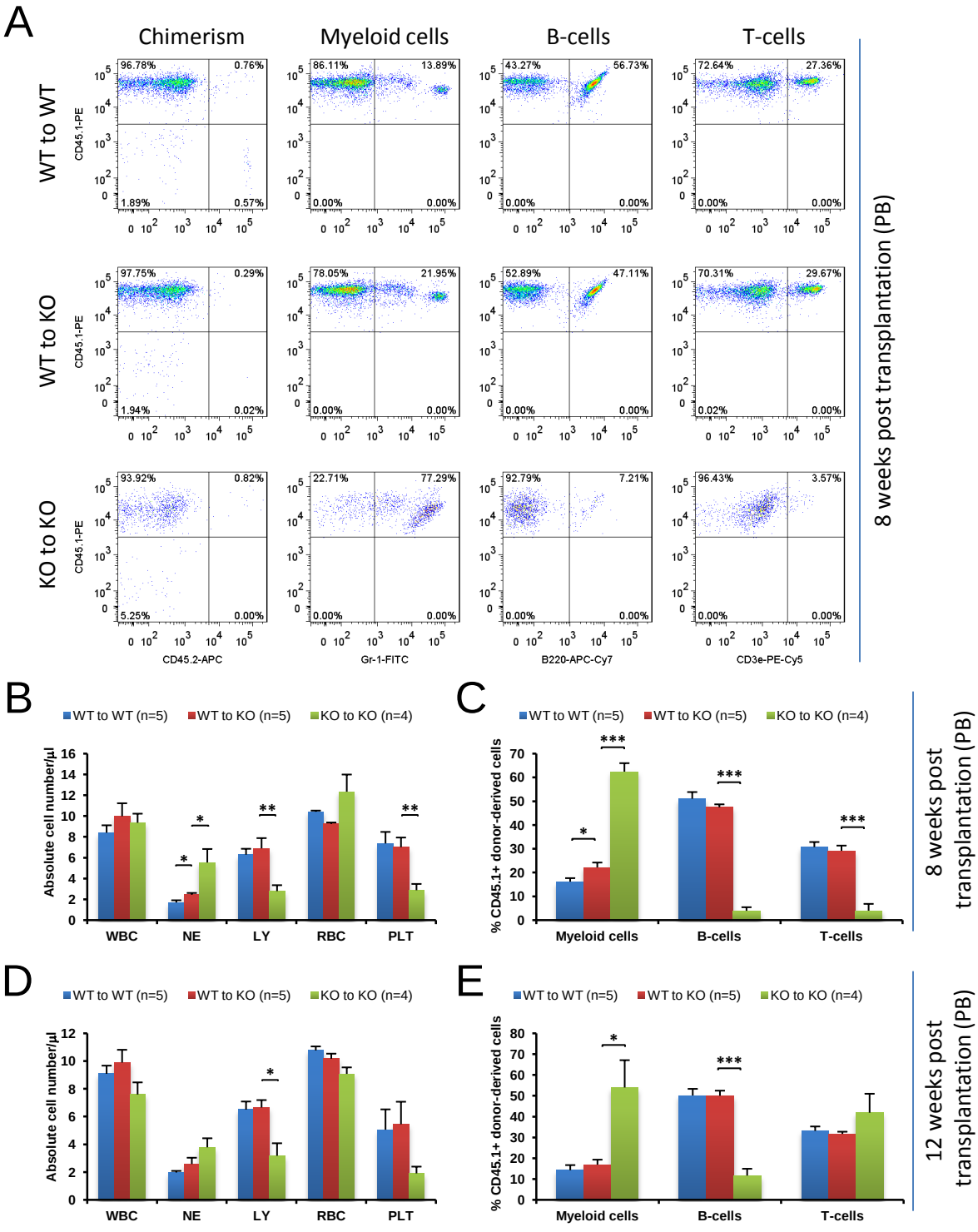
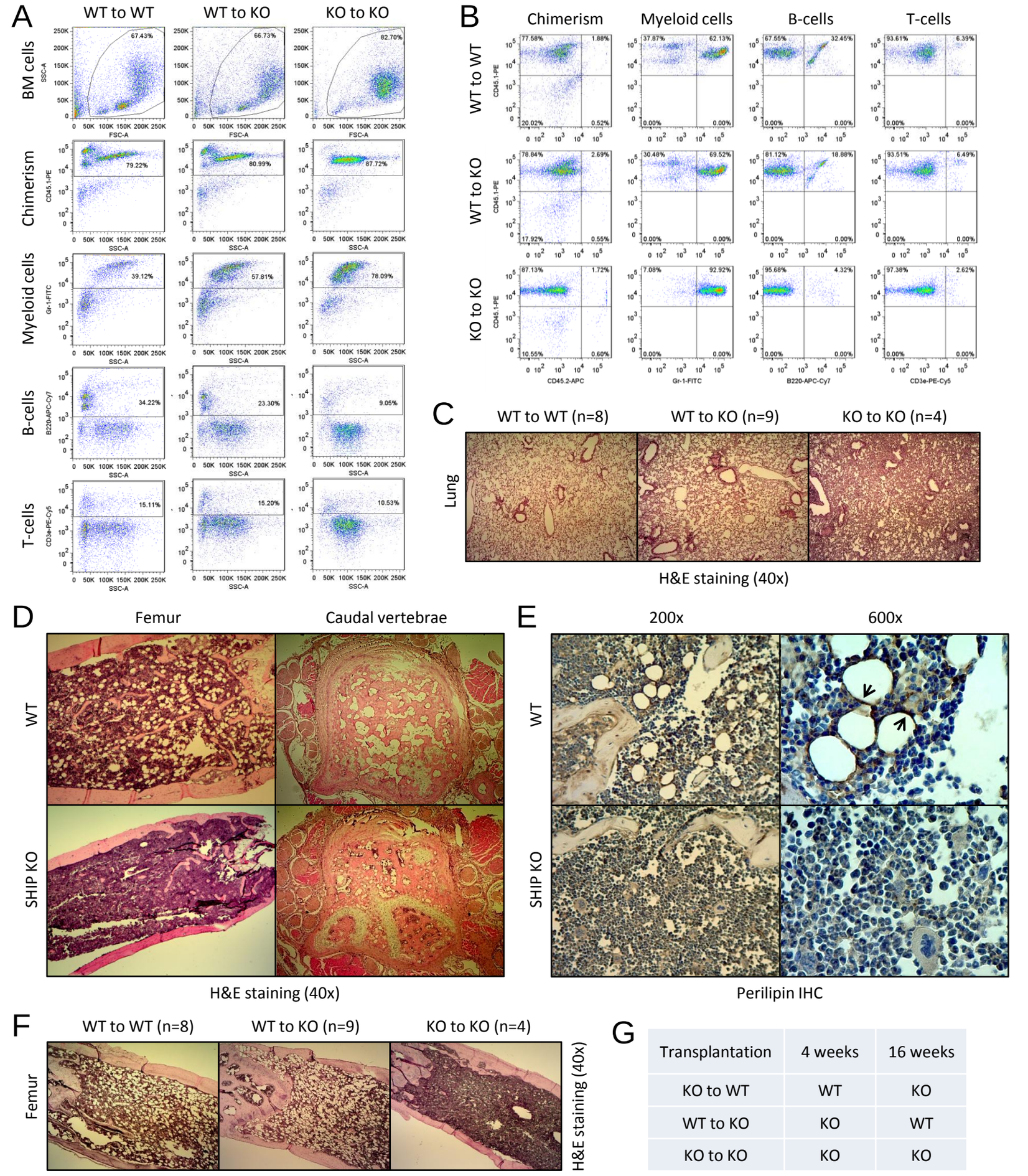


Figure S1, related to Figure 1. Transplanted WT whole bone marrow cells gradually converted SHIP KO hematopoietic phenotype to WT phenotype in the SHIP KO recipient mice. Experiments were conducted as shown in Figure 1. Peripheral blood (PB) cells were analyzed at 8 weeks (A, B and C) or 12 weeks (D and E) post transplantation. **(A-C)** Representative FACS analysis profile (A), absolute blood cell number (B) and donor-derived cell population (C), indicate transition to SHIP WT phenotype in the SHIP KO recipient PB at 8 weeks post transplantation. **(D-E)** Absolute blood cell number (D) and donor-derived cell population (E) indicate WT phenotype in the SHIP KO recipient PB at 12 weeks post transplantation. WBC: white blood cells (x 10^3); NE: neutrophils (x 10^3); LY: lymphocytes (x 10^3); RBC: red blood cells (x 10^6) and PLT: platelets (x 10^5). Data in (B), (C), (D) and (E) are mean ± SEM.*p<0.05, **p<0.01, ***p<0.001, two-tailed t-Test.



G

Transplantation	4 weeks	16 weeks
KO to WT	WT	KO
WT to KO	KO	WT
KO to KO	KO	KO

Figure S2, related to Figure 3. Transplanted WT BM cells converted SHIP KO to WT hematopoietic phenotype in the BM of recipient mice, reversed pathology of the lungs and restored adipogenesis in the BM of SHIP KO mice at 16 weeks post transplantation. Experiments were conducted as shown in Figure 1. Representative FACS profiles of **(A)** total and **(B)** donor-derived BM hematopoietic cells indicate conversion to WT hematopoietic phenotype of SHIP KO recipient mice. **(C)** H&E stained lung histological sections prepared from WT and SHIP KO recipients indicate normal cellularity in WT-to-KO transplant recipient lungs. **(D)** H&E stained femur (left) and caudal vertebrae (right) sections suggest SHIP KO BM contained less adipocytes. **(E)** Representative images of femur sections after immunohistochemical staining (IHC) with an antibody against adipocyte-specific perilipin (brown staining indicated by arrows), suggest SHIP deficient mice had no discernible adipocytes in the BM. **(F)** H&E stained femur histological sections prepared from WT and SHIP KO recipients indicate normal adipogenesis in the BM of WT-to-KO transplant recipients. **(G)** Summary of recipient hematopoietic phenotypes 4 or 16 weeks after whole BM transplantation.

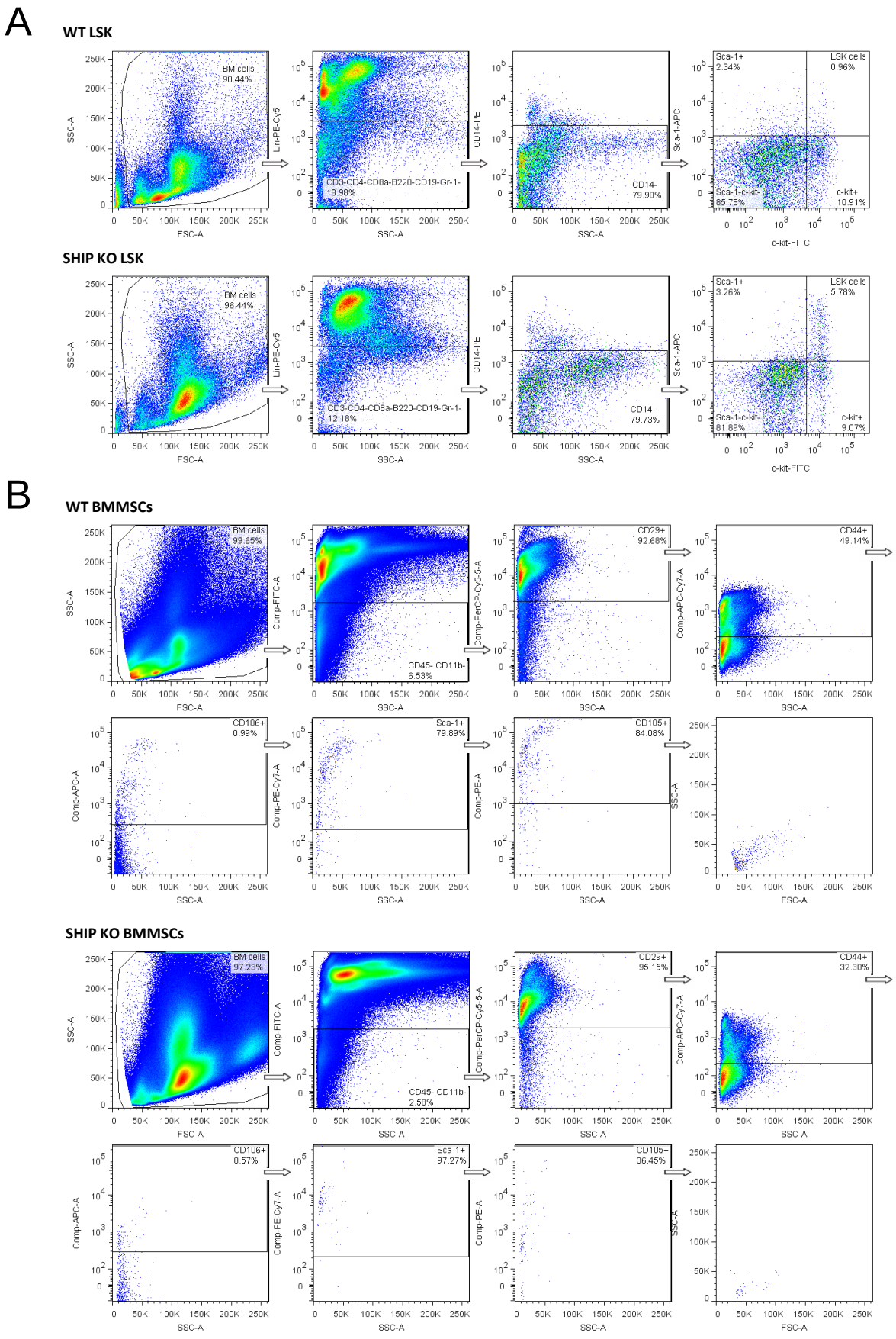
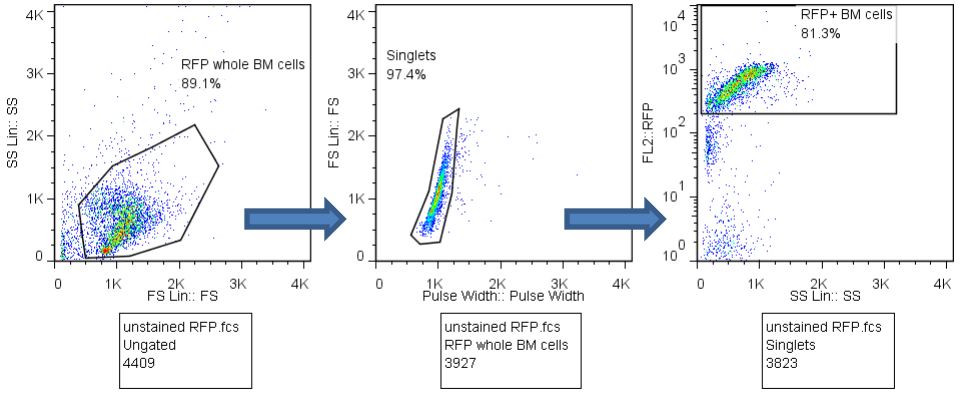


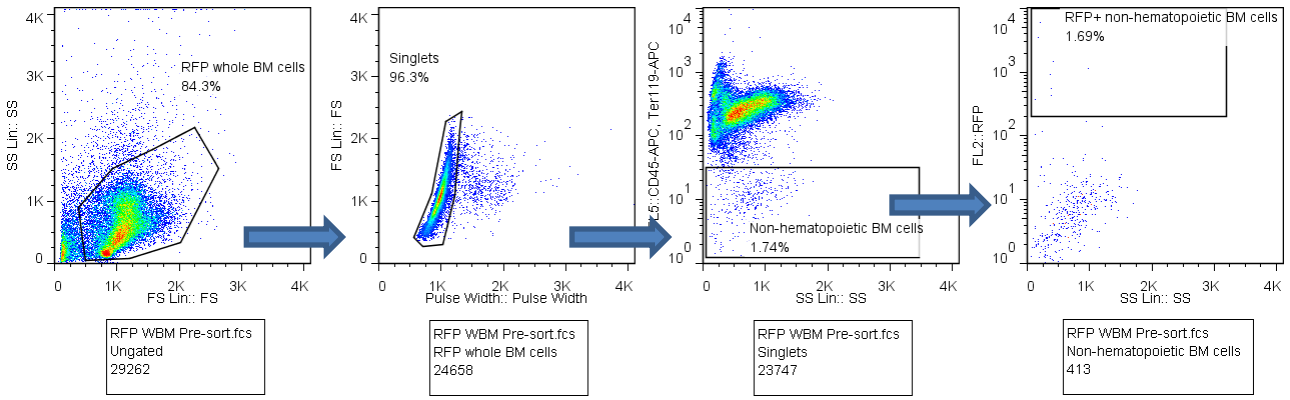
Figure S3, related to Figure 5. SHIP KO mouse BM contained more LSK cells and less multipotent stromal cells (BMMSCs) compared to WT mouse BM. Shown are representative FACS profiles of LSK cell population (**A**) and multipotent stromal cell population (**B**) in WT and SHIP KO mouse BM. Sequential gating is indicated by the arrows.

A

Unstained RFP mouse WBM cells:



RFP mouse WBM cells stained with CD45-APC and Ter119-APC:



B

GFP mouse WBM cells unstained and double stained:

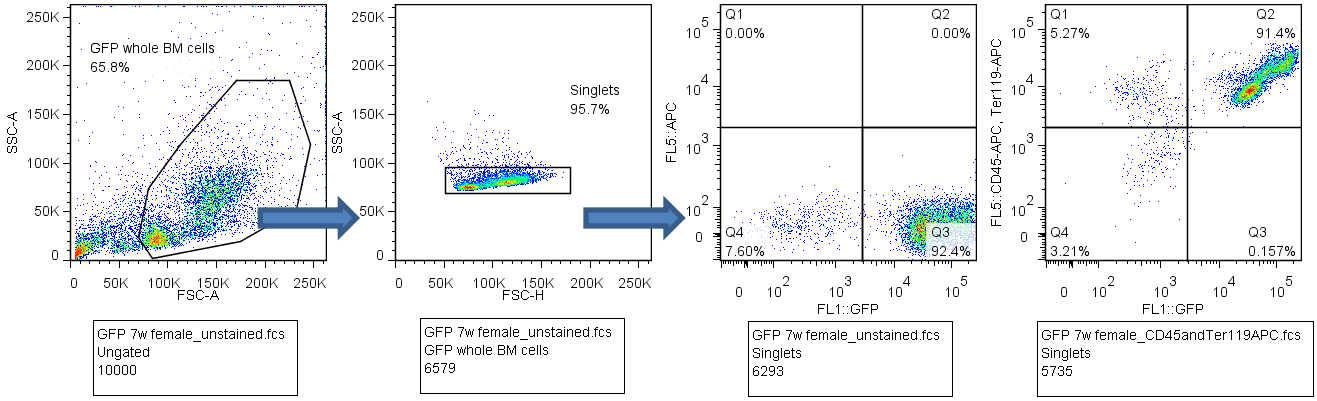


Figure S4, related to Figure 7. The non-hematopoietic BM cells in RFP and GFP mice were not fluorescent. (A) Representative FACS analysis profile of BM cells from RFP mice. **(B)** Representative FACS analysis profile of BM cells from GFP mice.

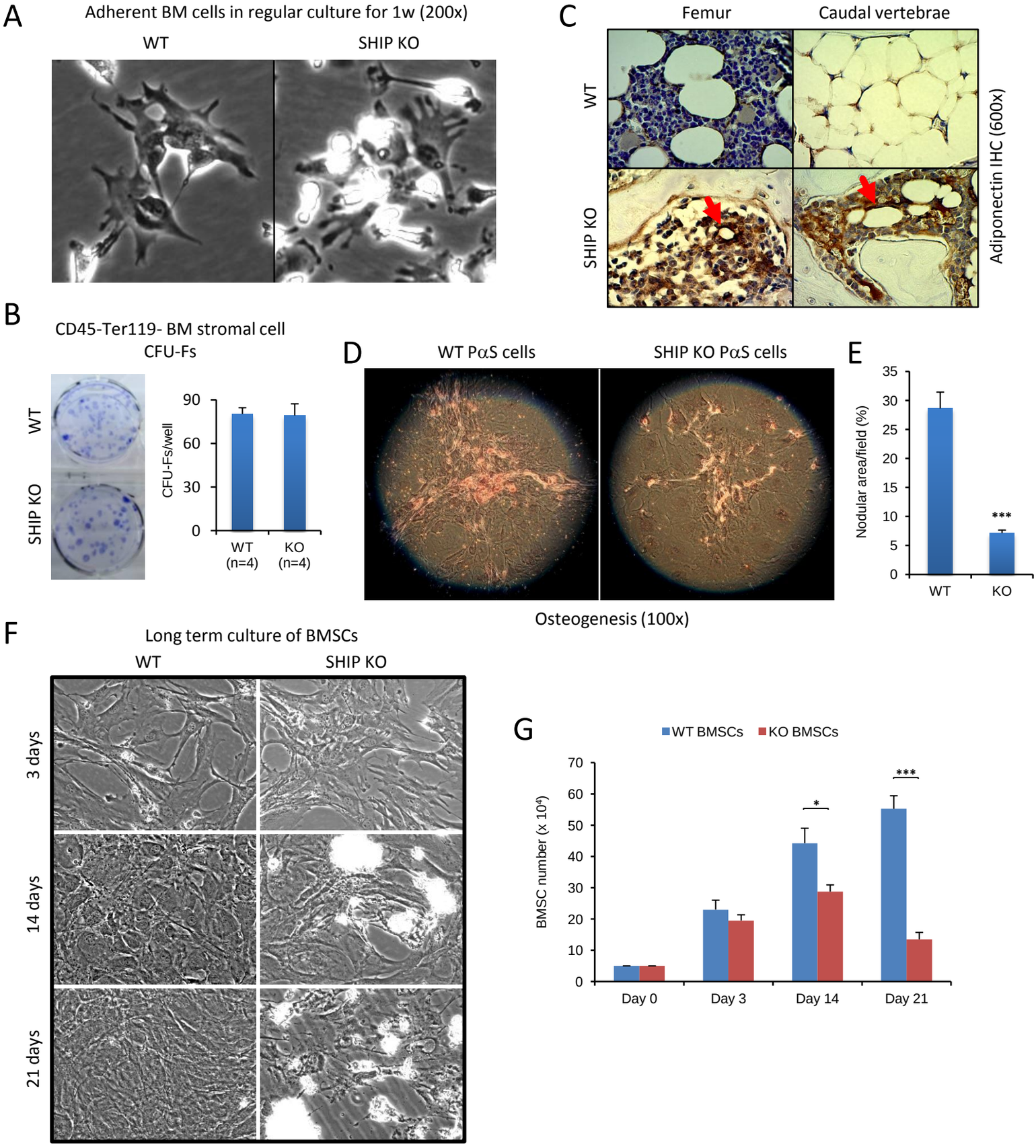


Figure S5, related to Figure 8. Characterization of SHIP deficient BM microenvironment. (**A**) More cells from SHIP KO mouse BM adhered to the plastic tissue culture dish compared to WT mouse BM. Shown are images of adherent BM cells in regular culture for 1 week. (**B**) WT and SHIP KO CD45-Ter119- BM stromal cells formed similar number of CFU-Fs. CD45-Ter119- BM cells from two pairs of femur and tibia were cultured for 1 week in a 6-well plate, and then stained with 0.05% crystal violet. Shown are representative images (left) and quantification (right). (**C**) SHIP deficient adipocytes expressed high level of adiponectin. Shown are representative femur and caudal vertebrae sections after IHC staining with specific antibodies against adiponectin (brown). Red arrows indicate adipocytes in SHIP KO mouse BM. (**D-E**) SHIP deficient PαS cells showed impaired ability to form osteoblastic nodule upon osteogenic induction. The osteogenic potential was assessed by Alizarin red. The stained nodular area per microscopic field (100x) was measured at 21d after osteogenic induction. (**F-G**) SHIP deficient CD45-Ter119- BM stromal cells (BMSCs) lost viability in long term culture. Shown are representative cell cultures starting with the same number of WT and SHIP KO BMSCs (5×10^4) in a 6-well plate. Data in (B), (E) and (G) are mean ± SEM. *p<0.05, ***p<0.001 by two-tailed t-Test.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mouse maintenance and genotyping. Animal procedures followed protocols approved by Institutional Animal Care and Use Committee at Children's Hospital Boston. The SHIP KO mice were purchased from the Jackson Laboratory and maintained on a mixed genetic background (129/C57BL/6). For mouse genotyping PCR, the WT and SHIP KO bands were detected using the primers (all synthesized by Integrated DNA Technologies): (A) 5' - CTG AAT GAA CTG CAG GAC GA - 3', (B) 5' - ATA CTT TCT CGG CAG GAG CA - 3', (C) 5' - GAA CCA TGG CAA CAT CAC C - 3', and (D) 5' - AGG AAG CTC CCG TCC TTG - 3'. SHIP heterozygous mice were then backcrossed with either CD45.2+ C57BL/6 mice or CD45.1+ C57BL/6 mice (both from Jackson). Over a period of two years of backcrossing, we generated both CD45.2+ SHIP KO mice in C57BL/6 background and CD45.1+ SHIP KO mice in C57BL/6 background. Examples of genotyping by FACS analysis of CD45.1+ SHIP KO mice (Figure 1B, cells from donor mice) and CD45.2+ SHIP KO mice (Figure 1B, cells from recipient mice) are provided. Every donor and recipient mouse used in the transplantation experiments was carefully genotyped for its CD45.1 and/or CD45.2 expression before actual experiment. RFP mice were purchased from Jackson Laboratory; GFP mice were obtained from Carla Kim's lab (Stem Cell Program, Children's Hospital Boston). The numbers of mice analyzed per group for these transplant experiments are indicated in the figures; the data presented are a combination of three sets of separate experiments.

FACS analyses. For LSK cell and multipotent stromal cell analysis, BM cells were prepared by spinning femurs and tibias. Red blood cells were then lysed using ACK lysing buffer (Invitrogen). After washing with PBS containing 2% fetal calf serum, cells were first blocked with CD16/32 for 30min on ice before specific antibodies were added and incubated for 1h on ice. For hematopoietic lineage determination following anti-mouse antibodies were used: PE-Cy5-labeled CD3, CD4, CD8a, B220, CD19 and Gr-1, PE-CD14, APC-Sca-1, FITC-c-kit, and FITC-CD11b. For multipotent stromal cell analysis following anti-mouse antibodies were used: FITC-CD45, FITC-CD11b, PerCP-Cy5.5-CD29, APC-Cy7-CD44, APC-CD106, PE-Cy7-Sca-1, and PE-CD105. For transplantation recipient analysis, PB was collected by retro-orbital bleeding

and Hemavet (Drew Scientific) was used to determine absolute blood cell number in PB. BM cells were collected by crushing femurs and tibias with mortar and pestle. After red blood cell lysis, following antibodies were added to determine chimerism and hematopoietic cell lineage: PE-CD45.1, APC-CD45.2, FITC-Gr-1, APC-Cy7-B220, and PE-Cy5-CD3e. These fluorophore labeled anti-mouse antibodies were purchase from BD Pharmingen, eBioscience or Biolegend. Analyses were performed using a BD FACSCanto II and software FlowJo 7.6.1.

BM cell transplantation. Age-matching CD45.2+ C57BL/6 and CD45.1 (B6.SJL-*Ptprc*^{a}/BoyAiTac) mice were purchased from Taconic (Hudson, NY). Donor whole BM cells were prepared by spinning femurs and tibias under sterile conditions, and red blood cells were lysed using ACK lysing buffer. BM cell transplantations were performed following twice 5.2 Gy 3h apart lethal γ-irradiation of recipient mice. For transplantation experiments: WT-to-WT, WT-to-KO, KO-to-WT and KO-to-KO, 3 x 10^6 BM cells were transplanted into each recipient mouse via tail vein injection. The same protocol was used to transplant whole BM cells from RFP into SHIP KO mice. For experiments in which WT hematopoietic cells combined with SHIP KO non-hematopoietic cells were transplanted into SHIP KO mice, each recipient received 3 x 10^6 WT mouse BM CD45+ and/or Ter119+ hematopoietic cells and 3 x 10^4 SHIP KO mouse BM CD45- and Ter119- non-hematopoietic cells, which were obtained by flow cytometry sorting (Beckman Coulter MoFlo Legacy with software Summit version 4.3). For experiments in which WT hematopoietic cells alone were transplanted into SHIP KO mice, each recipient received 3 x 10^6 CD45+ and/or Ter119+ hematopoietic cells from WT mouse BM. For experiments in which WT hematopoietic cells and WT PαS cells were combined to transplant into lethally irradiated WT or SHIP KO mice, each recipient received 3 x 10^6 WT BM CD45+ and/or Ter119+ hematopoietic cells and 5 x 10^3 WT PαS cells. Quantification of femoral adipocytes of WT-to-WT, WT-to-KO and KO-to-KO transplant recipients was performed by manual counting of H&E stained sections under a microscope with 40x magnification and quantification of lung cellularity of the transplant recipients was performed using the NIH ImageJ program.

Immunohistochemistry. Mice were perfused with 10% neutral buffered formalin (Sigma) immediately following euthanasia by carbon dioxide asphyxiation. Spleen, lung and femurs

were fixed for histology. Bones were decalcified with formic acid/sodium formate mix, paraffin embedded, sectioned and stained with H&E reagents. Perilipin and adiponectin immunohistochemistry was performed using standard protocols and reagents from Vector Labs. Briefly, slides had paraffin removed in xylene and were rehydrated through ethanol. Slides were then washed in PBS followed by inactivation of endogenous peroxidase by incubation with 0.3% H_2O_2 in methanol. After blocking with normal serum, the slides were incubated with primary antibody (rabbit anti-perilipin from Abcam or goat anti-mouse adiponectin from R&D Systems) for 30 min at room temperature. Next, they were washed three times with PBS followed by incubation with biotinylated secondary antibodies (Vectastain ABC Kit) and detection using a DAB Substrate Kit (Vector Labs). All samples were counterstained with Hematoxylin (Vector Labs).

Determination of non-hematopoietic chimerism in the BM of SHIP KO recipients. We applied three different approaches to quantify non-hematopoietic chimerism in the BM of SHIP KO recipients: To detect WT SHIP DNA in SHIP KO recipients; to detect male DNA in female recipients and to detect RFP reporter gene in non-RFP SHIP KO recipients. Conventional PCR genotyping was performed to detect the presence of WT and SHIP KO bands in hematopoietic and non-hematopoietic cells from the BM of SHIP KO recipients. Quantitative real-time PCR was performed using a CFX96 Thermal Cycler and iQ SYBR Green Supermix or iQ Multiplex Powermix from Bio-Rad. The sequence of murine WT SHIP DNA primers were as follows (http://jaxmice.jax.org/strain/003534.html): forward, 5' - GAA CCA TGG CAA CAT CAC C - 3'; reverse, 5' - AGG AAG CTC CCG TCC TTG - 3'. The PCR reactions were performed in a volume of 50 μl and contained 25 μl of the SYBR Green Supermix, 320 nM forward and reverse primers. Reactions were incubated at 94^{o}C for 3 min, and then amplified for 36 cycles, each cycle comprised of an incubation step at 94^{o}C for 30 sec followed by 58^{o}C for 1 min and 72^{o}C for 1 min; and the reactions were completed with a step at 72^{o}C for 2 min. Standard curves were generated by serially diluting genomic DNA from BM cells of WT mice into that of KO mice. The sequence of murine Y-chromosome specific DNA primers and dual-labeled probe were as follows (McBride et al., 2003): forward, 5' - TTT TGC CTC CCA TAG TAG TAT TTC CT - 3'; reverse, 5' - TGT ACC GCT CTG CCA ACC A - 3'; TaqMan probe, 5' - (FAM) - AGG

GAT GCC CAC CTC GCC AGA - (Black Hole Quencher-1) - 3'. Conditions for real-time PCR to quantify male DNA were the same as described by McBride et al. The sequence of RFP DNA primers were as follows: forward, 5' - AGT TCA AGT CCA TCT ACA TGG CCA AGA AGC CCG - 3'; reverse, 5' - TCG TAC TGC TCC ACG ATG GTG TAG TCC TCG TT - 3'. The PCR reactions were performed in a volume of 25 μl and contained 12.5 μl of the SYBR Green Supermix, 800 nM forward and reverse primers. Reactions were incubated at 94^{o}C for 5 min, and then amplified for 35 cycles, each cycle comprised of an incubation step at 94^{o}C for 30 sec followed by 61^{o}C for 1 min and 72^{o}C for 45 sec; and the reactions were completed with a step at 72^{o}C for 2 min. Standard curves were generated by serially diluting genomic DNA from BM cells of RFP mice into that of non-RFP mice. All primers were synthesized by Integrated DNA Technologies. DNA concentrations were measured using Nanodrop 2000C (Thermo Scientific).

Immunofluorescent staining and laser scanning cytometry of BM sections. Mice were perfused post-mortem with 10 ml paraformaldehyde-lysine-periodate (PLP) fixative through the vena cava to achieve rapid *in situ* fixation and optimal preservation of the bone marrow tissue. Femoral bones were isolated, fixed in PLP for 4-8 hours, rehydrated in 30% sucrose/PBS for 48 hours and snap frozen in OCT (TissueTek). Cryosections of non-decalcified whole longitudinal femoral bones were obtained using a Leica Cryostat and the Cryojane tape transfer system (Leica Microsystems). Bone marrow sections were stained with rabbit anti-Laminin (Cat. L9393, Sigma Aldrich) and goat anti-c-kit (R&D systems) polyclonal antibodies. As secondary antibodies, DyLight 488-donkey anti-goat IgG and DyLight-649 donkey anti-rabbit IgG (Jackson Immunoresearch) were employed. DAPI (Invitrogen) staining was used for nuclear detection and sections were mounted with Vectashield mounting medium for immunofluoresence (Vector Labs). High resolution images of whole longitudinal immunostained femoral sections were obtained with an iCys Research Imaging Cytometer (Compucyte Corporation) equipped with four laser lines (405, 488, 561 and 633 nm) and four PMT detectors with bandpass emission filters at 450/,40, 521/15, 575/50 and 650LP.

PαS cell isolation and cultivation. PαS cells were isolated and cultured using reagents and methods as described by Morikawa et al. (Morikawa et al., 2009). Briefly, Femurs and tibias

from WT or SHIP KO mice were dissected and crushed with mortar and pestle. The bone fragments were gently washed once in HBSS supplemented with 2% fetal bovine serum (FBS), 10mM Hepes, and 1% penicillin/streptomycin (P/S). The cell suspension filtered through a 70μm cell strainer (BD Falcon) was discarded. The bone fragments were incubated for 1h at 37^{o}C in 20ml DMEM (Invitrogen) containing 0.2% collagenase (Wako Chemicals), 10mM Hepes, and 1% P/S. The cell suspension was filtered through a cell strainer to remove debris and bone pieces, and cells were collected by centrifugation at 280g for 7min at 4^{o}C. The pellet was immersed in 1ml water for 5-10s to burst the red blood cells, after which 1ml of 2x PBS containing 4% FBS was added, and the suspension was filtered through a cell strainer. The cells were suspended in ice-cold HBSS containing supplements as above at 1-5x 10^7 cell/ml, and stained for 30min on ice with the following antibodies APC-PDGFRα (APA5), FITC-Sca-1 (Ly6A/E), PE-CD45 (30-F11) and PE-Ter119 (Ter-119) (all from eBioscience). Flow cytometry analysis and sorting were performed on a Beckman Coulter MoFlo Legacy with software Summit version 4.3. The CD45-, Ter119-, PDGFRα+, and Sca-1+ (PαS) cells were allowed to adhere to the plastic surface of a 25cm^2 tissue culture flask (Falcon 3081) for 48h without disturbance in α-MEM medium (Invitrogen) supplemented with 10% non-heat-inactivated FBS (Hyclone) (Kuznetsov et al., 2009), 10% horse serum (Sigma), 1x L-Glutamine (Invitrogen) and 1% P/S, as described by Peister et al. (Peister et al., 2004).

Characterization of PαS cells. Differentiation of PαS cells was carried out according to published methods (Peister et al., 2004). Briefly, in a 6-well plate 2 x 10^5 cells were seeded in each well. On the next day, the cells were washed once with PBS, and 2 ml adipogenic or osteogenic medium was added to each well. Adipogenesis was induced by culturing PαS cells in complete α-MEM medium as described above, supplemented with 5 μg/ml Insulin, 50 μM Indomethacin, 1 μM Dexamethasone, and 0.5 μM 3-isobutyl-1 methylxanthine (IBMX). After two weeks, the cells were fixed with 10% Formalin and stained with 0.5% Oil Red O in Methanol. For osteogenesis, the PαS cells were incubated in complete α-MEM medium supplemented with 20 mM β-glycerol phosphate, 50 ng/ml thyroxine, 1 nM Dexamethasone and 0.5 μM ascorbate 2-phosphate, with medium change three times per week. At the end of 3 weeks, the cells were fixed with 10% Formalin and stained with Alizarin Red. Quantification of

nodular area/field was performed with the NIH ImageJ program. All reagents were obtained from Sigma.

Apoptosis of PαS cells was assessed by TUNEL staining using a TACS 2 TdT-Fluor in situ Apoptosis Detection Kit (Trevigen). Briefly, 1x 10^5 PαS cells were seeded in a glass bottom (No. 1.0 coverglass, 0.13-0.16mm thick) 35mm culture dish (MatTek P35G-1.0-14-C). Two days later, medium was removed and the cells were washed once with PBS and fixed with 3.7% buffered formaldehyde for 10 min at room temperature. The cells on the coverglass were then washed once with PBS followed by incubation with 50 μl Cytonin for 60 min at room temperature. After two washes in deionized water, the cells were immersed in 1x TdT labeling buffer for 5 min followed by incubation with 50μl labeling reaction mix at 37^{o}C for 1h. The reaction was stopped by 1x TdT stop buffer followed by two washes with deionized water. The sample was then covered with 50μl of strep-fluor solution for 20 min at room temperature. The cells were washed twice in PBS and then visualized under a fluorescence microscope (Olympus 1X71). Equal numbers of WT and SHIP KO PαS cells were used in the TUNEL assays, which also included both positive and negative controls as per manufacturer's instruction. For irradiation experiments, the cells received one dose of 10.4 Gy γ-irradiation.

For Western blotting, 2x 10^5 whole bone marrow cells or PαS cells from passages 4 to 6 were lysed in 200μl 1x SDS-loading buffer. Proteins were separated on a 4-15% Mini-PROTEAN TGX gel (Bio-Rad), and then transferred onto a PVDF membrane. After blocking with PBS containing 0.1% Tween-20 and 5% BSA for 1h at room temperature, the membrane was incubated with appropriately diluted primary antibodies at 4^{o}C overnight with gentle shaking. On the next day, after two washes with PBS containing 0.1% Tween-20, the membrane was incubated with corresponding secondary antibodies conjugated with horseradish peroxidase (1:1000 in PBS containing 0.1% Tween-20) for 1h at room temperature. Membrane bound peroxidase was visualized by exposing to an Amersham Hyperfilm (GE Healthcare) after incubation with Amersham ECL Plus detection reagents (GE Healthcare). Following antibodies were used in western blotting: goat anti-mouse adiponectin (R&D Systems), anti-mouse PPARγ (clone E-8, Santa Cruz), anti-phospho-PPARγ (Ser82) (clone AW504, Upstate), anti-phospho-PPARγ (Ser112) (Millipore), anti-phospho-retinoblastoma protein (Ser608) (clone 51B7, Abcam), anti-FOXO1 (clone C29H4, Cell Signaling), anti-phospho-FOXO1 (Thr24)/FOXO3a

(Thr32) (Cell Signaling), anti-AKT (Cell Signaling), anti-phospho-AKT (Ser473) (Cell Signaling), anti-caspase-3 (Cell Signaling) and anti-β-actin (Cell Signaling).

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