Supplementary Materials

Supplementary Methods

Mouse HSC culture

Indicated numbers of BM $Lin^-Sca\text{-}1^+Kit^+Flk2^-CD34^-$ cells were isolated from 8-12 week old Sclpfn1 or wild-type C57BL/6 mice and were plated into wells of a U-bottom 96-well plate (3799; Corning) with StemSpan serum-free medium (StemCell Technologies) supplemented with 10 μg/ml heparin (Sigma), 10 ng/ml mouse SCF (R&D Systems), 20 ng/ml mouse TPO (R&D Systems), and 10 ng/ml human FGF-1 (Invitrogen). Cells were cultured at 37 ^oC in 5% CO_2 and the normal level of O_2.

Flow cytometry

Donor BM cells were isolated from 8-12 week old control or Sclpfn1 mice. $Lin^-Sca\text{-}1^+Kit^+Flk2^-CD34^-$ cells (LT-HSCs) were isolated by FACS as described [12]. For analysis of repopulation of mouse HSCs, peripheral blood cells of recipient CD45.1 mice were collected by retro-orbital bleeding, followed by lysis of red blood cells, and staining with anti-CD45.2, anti-CD45.1, and lineage markers as described [12]. The "percent repopulation" shown in all figures was based on the staining results of anti-CD45.2-FITC and anti-CD45.1-PE or GFP and anti-CD45.1-PE. Antibodies were all purchased from BD Biosciences. In all cases FACS analysis of the above listed lineages was also performed to confirm multi-lineage reconstitution as previously described [1]. The Hoechst 33342 and pyronin Y staining and BrdU incorporation were performed as described [12]. Hoechst, pyronin Y, and BrdU were purchased from Sigma.

Competitive reconstitution analyses

The indicated numbers of mouse CD45.2 donor cells were mixed with 2 x 10^5 freshly isolated CD45.1 competitor BM cells and the mixture was injected intravenously via the retro-orbital route into each of a group of 6-8 week old CD45.1 mice previously irradiated with a total dose of 10 Gy. To measure reconstitution, peripheral blood was collected at the indicated time points post-transplant and the presence of $CD45.1^+$ and $CD45.2^+$ cells were measured as described [13,14].

Measurement of ^{13}C lactate production

The metabolic assays were performed essentially as we described [1,15]. Lactate production was measured with gas chromatography-mass spectrometry as described previously [1]. Cells were cultured for 12 hr in a medium supplemented with 10 mM D-[1-^{13}C, 6-^{13}C]-glucose (Cambridge Isotope Labs) to allow up to half of the glucose-derived lactate pool to be labeled. The samples were analyzed for lactate abundance. The final results are presented as nmol ^{13}C-lactate/nmol ATP.

ATP assay

ATP standard curves were prepared using ATP concentrations between 10^{-6}-10^{-12} M. ATP standards (50 μl) and 50 μl cell lysates were quantified using ATP Bioluminescence Assay Kit HS II (Roche) using Fluostar Optima plate reader (BMG Labtech) following the manufacturer's instructions. Data were normalized to cell counts.

Oxygen consumption

Oxygen consumption was determined using the BD Oxygen Biosensor System according to manufacturer's recommendations. Control and Sclpfn1 $Lin^{-}Sca\text{-}1^{+}Kit^{+}Flk2^{-}CD34^{-}$ cells were isolated as described above. Equal numbers of cells (5 x 10^4 cells/well in a 50 µl volume) were incubated up to 6 hours in the provided 384-well plate prior to measurement of fluorescence with a Fluostar Optima plate reader (BMG Labtech). Culture media without cells and media without sodium sulfite (100 mM) were used as negative and positive controls, respectively. Oxygen consumption is presented as relative units.

Homing

Homing assays were performed as described [12,13]. Briefly, BM cells were labeled with 5- and 6-carboxyfluorescein succinimidyl ester (CFSE), and 1-2 x 10^7 cells were transplanted into indicated strains of lethally irradiated mice. After 16 hours, the total number of $CFSE^{+}$ LSKFC cells in the BM, spleen, and liver were determined by flow cytometry. Antibodies were all purchased from BD Biosciences.

Colony assays

Normal BM cells were diluted to 2 x 10^5 cells/ml in IMDM with 2% FBS and were then seeded into methylcellulose medium M3334 (StemCell Technologies) for analysis of CFU-E colony formation, 2 x 10^4 cells/ml into M3434 (StemCell Technologies) for CFU-GM and BFU-E colony formation analysis, or 2 x 10^4 cells/ml into M3630 (StemCell Technologies) for CFU-Pre-B colony formation assays as described previously [13]. CFU

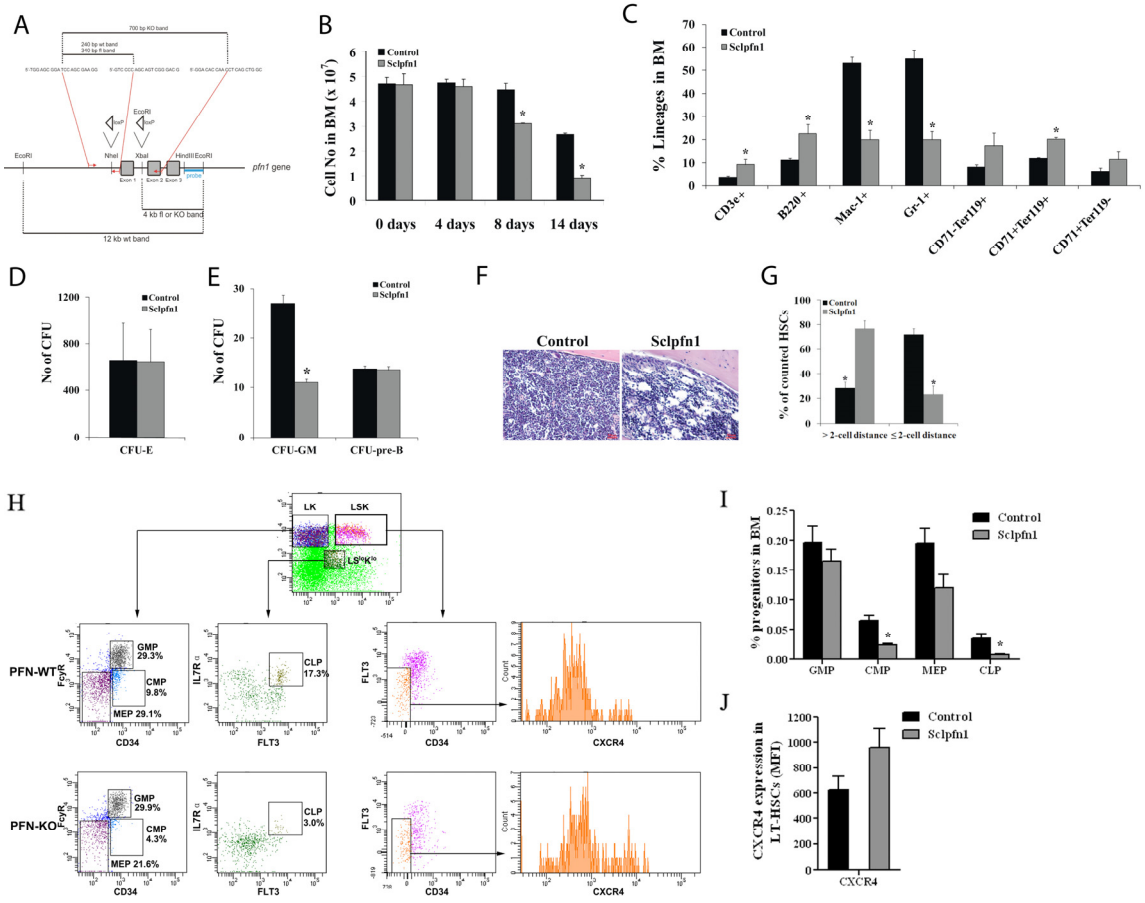
was scored after 2 days of culture. CFU-GM and BFU-E were scored after 10 and 7 days of culture respectively.

Retrovirus infection

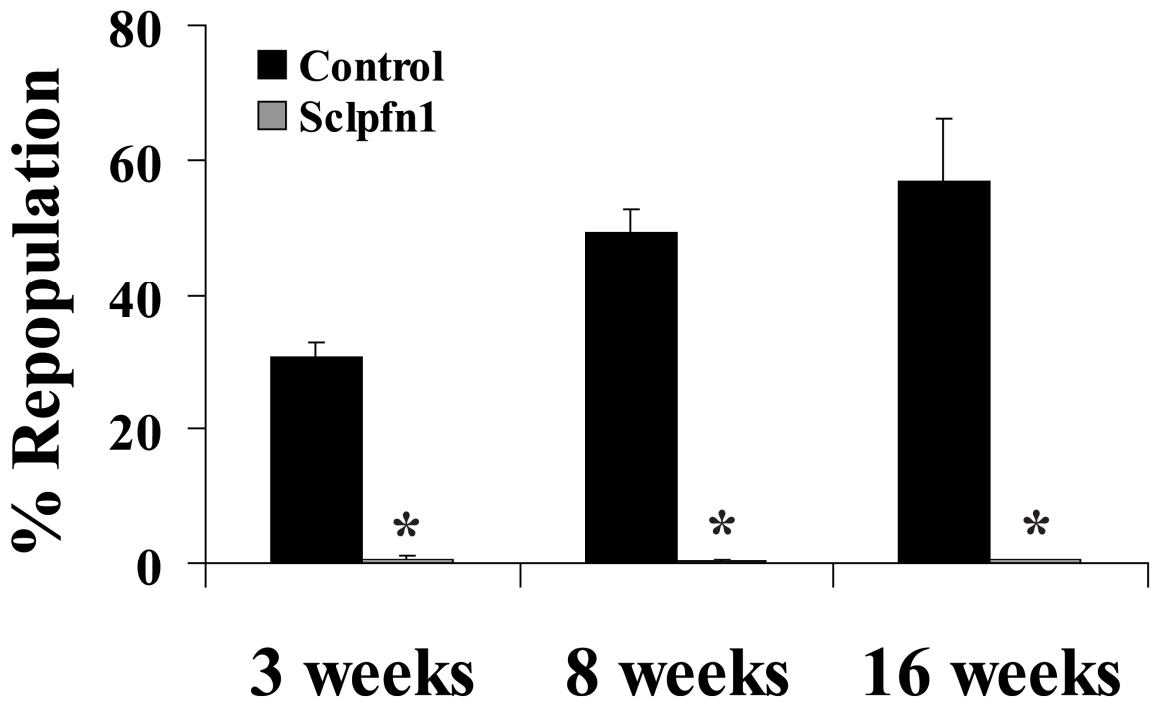
The pfn1 retroviral plasmids, including MSCV-WT-IRES-GFP, MSCV-R74E-IRES-GFP, MSCV-Y6D-IRES-GFP, and MSCV-H133S-IRES-GFP with PCL-ECO (2:1), were transfected using lipofectamine 2000 (Invitrogen) into 293T cells. The resulting retroviral supernatant was collected 48-72 hours later and was used for infection as described [12].

Quantitative RT-PCR

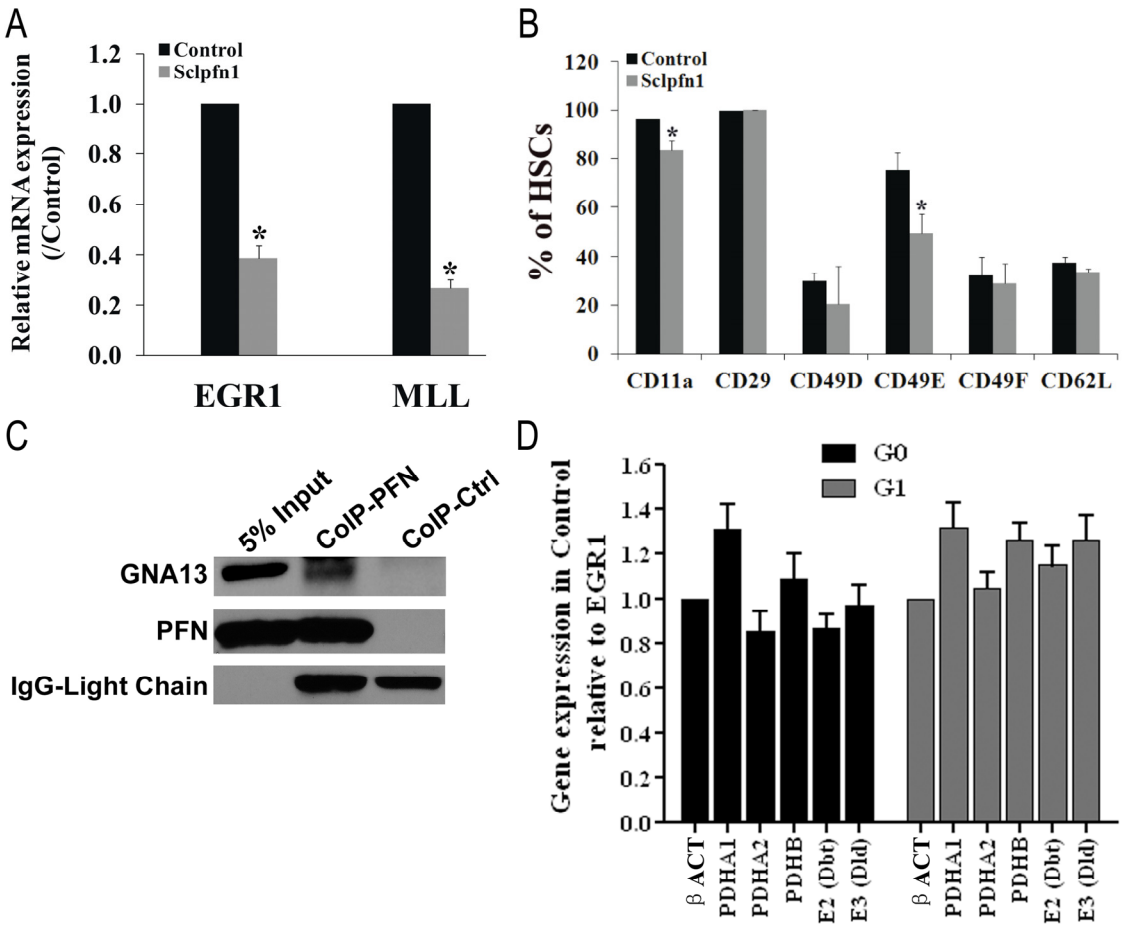
Total RNA was isolated from FACS-collected BM $Lin^{-}Sca\text{-}1^{+}Kit^{+}Flk2^{-}CD34^{-}$ cells. The mRNA level in each population was normalized to the level of β-actin RNA transcripts present in the same sample as described [12]. Primers were described in sTable 2.


A
700 bp KO band
240 bp wt band
340 bp fl band
EcoRI
loxP
loxP
EcoRI
NheI
XbaI
HindIII EcoRI
pfn1 gene
Exon 1
Exon 2
Exon 3
probe
4 kb fl or KO band
12 kb wt band
B
Control
Sclpfn1
Cell No in BM (x 10^7)
0 days
4 days
8 days
14 days
C
Control
Sclpfn1
% Lineages in BM
CD3e+
B220+
Mac-1+
Gr-1+
CD71-Ter119+
CD71+Ter119+
CD71+Ter119-
D
Control
Sclpfn1
No of CFU
CFU-E
E
Control
Sclpfn1
No of CFU
CFU-GM
CFU-pre-B
F
Control
Sclpfn1
G
Control
Sclpfn1
% of counted HSCs
> 2-cell distance
≤ 2-cell distance
H
LK
LSK
LS⁺K⁺
PFN-WT
GMP 29.3%
CMP 9.8%
MEP 29.1%
CLP 17.3%
FcγR
IL7R
FLT3
CD34
CXCR4
Count
PFN-KO
GMP 29.9%
CMP 4.3%
MEP 21.6%
CLP 3.0%
I
Control
Sclpfn1
% progenitors in BM
GMP
CMP
MEP
CLP
J
Control
Sclpfn1
CXCR4 expression in LT-HSCs (MFI)
CXCR4

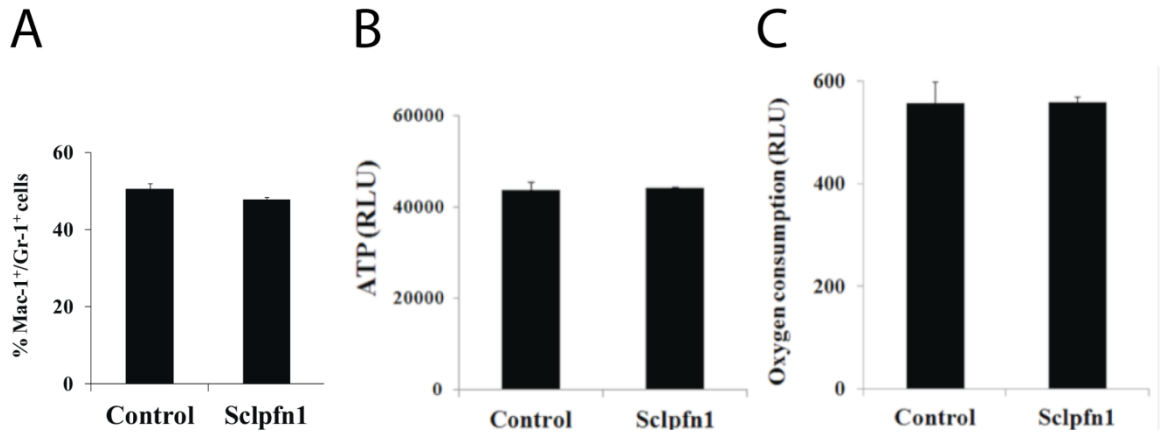
sFig. 1. Schematic of the primers for PCR-genotyping and hematopoietic alteration in BM of Sclpfn1 mice. (A) By using PCR, WT, fl or KO bands can be detected as 240bp, 340bp or 700bp, respectively. (B) Total BM cell numbers are shown at different time points after tamoxifen treatment in control and Sclpfn1 mice ($n = 5$; *, $p < 0.05$). (C) The major lineages of hematopoietic cells in BM of control and Sclpfn1 mice were quantified by flow cytometry analysis as T cells ($CD3^+$), B cells ($B220^+$), myeloid cells ($Mac\text{-}1^+$ or $Gr\text{-}1^+$), and erythroid cells ($CD71^-Ter119^+$, $CD71^+Ter119^+$, and $CD71^+Ter119^-$). (D-E) Total BM cell populations were plated in methylcellulose medium M3434 (StemCell Technologies) for quantification of CFU-GM, CFU-GEMM, and BFU-E colonies, in M3334 for quantification of CFU-E colonies, and in M3630 for quantification of CFU-pre-B colonies ($n = 3$; *, $p < 0.05$). (F) Loss of cellularity of BM after pfn1 deletion. Control and Sclpfn1 mice were treated with Tamoxifen for 14 days. The tibia and femur were decalcified and embedded in paraffin. Sections were further analyzed after HE staining and the cell density in Sclpfn1 bone marrow was dramatically decreased. Scale bar = 50 μM. (G) Dislocation of HSCs in bone marrow niches in pfn1-null mice. Control and Sclpfn1 mice were treated with Tamoxifen for 14 days. BM was stained to reveal $CD150^+CD48^-CD41^-Lin^-$ HSCs, $MECA\text{-}32^+$ sinusoidal endothelial cells (HSC niche). Only 23.35% of pfn1-null HSCs were adjacent to $MECA\text{-}32^+$ cells (≤ 2 cell distances), much less than that of control HSCs (71.7%). By contrast, 76.65% of pfn1-null HSCs did not locate in the correct niches (> 2 cell distances), which was much higher than controls (*, significantly different between two groups; $p < 0.05$, $n = 3$; total 60 HSCs were counted for each group). (H-J) Progenitors, including CMP (common myeloid progenitor), CLP (common lymphoid progenitor), GMP (granulocyte/monocyte progenitors) and MEP (megakaryocyte/erythrocyte progenitors), in BM of control and Sclpfn1 mice were analyzed, and surface expression of CXCR4 on LT-HSCs ($Lin^-Sca\text{-}1^+cKit^+Flk2^-CD34^-$) were measured by FACS. H), Representative FACS plots, I) Percentage of progenitors in total BM, J), CXCR4 expression on LT-HSCs (MFI, Median Fluorescence Intensity). *, significantly different between two groups; $p < 0.05$, $n = 3$.



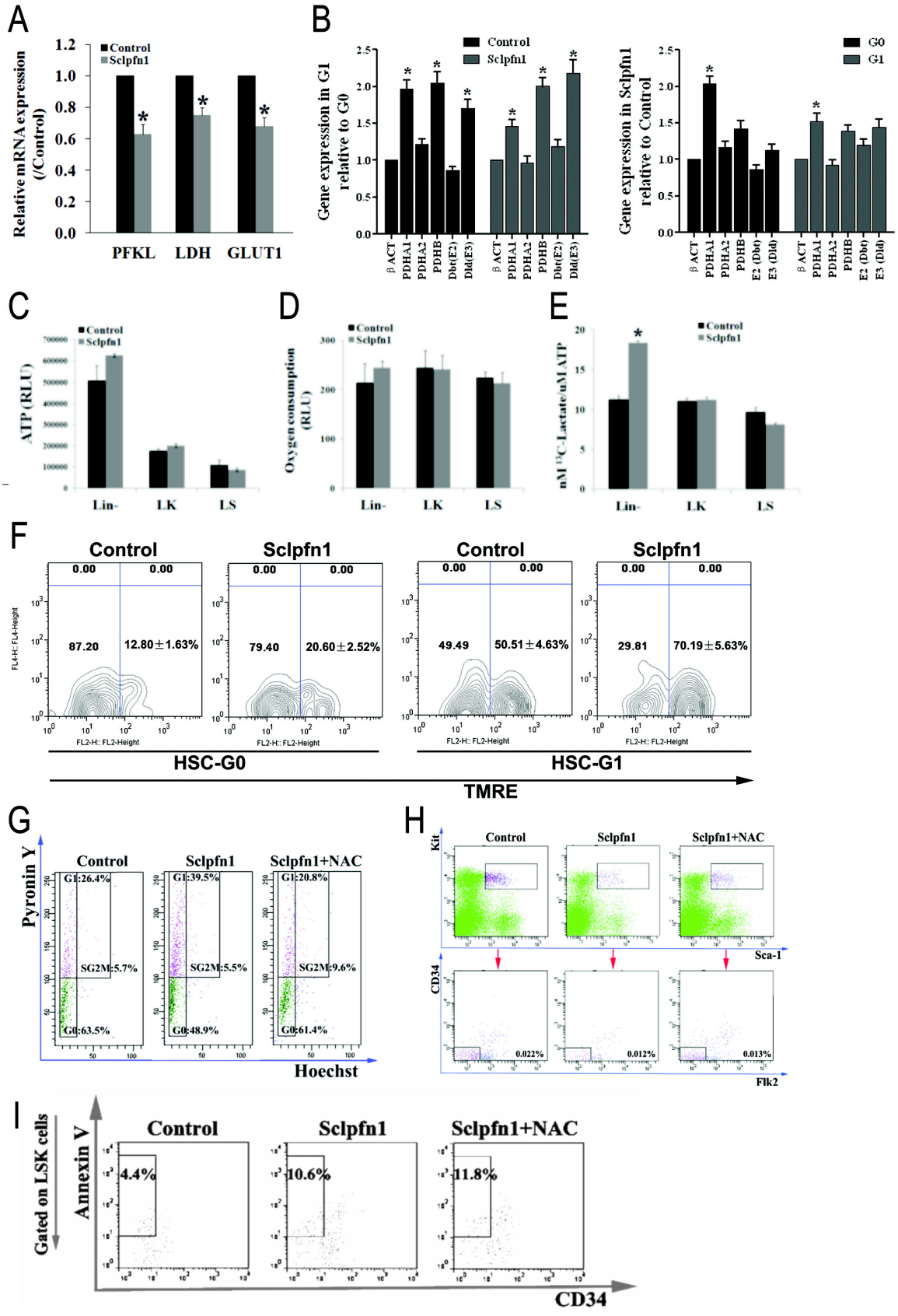
sFig. 2. Deletion of pfn1 in HSCs results in decreased repopulation. Control or Sclpfn1 donor cells (2 x 10^5) treated for 8 days with tamoxifen were co-transplanted along with 2 x 10^5 freshly isolated CD45.1 bone marrow competitors into lethally irradiated CD45.1 wild-type recipient mice. The engraftments at 3, 8, and 16 weeks post-transplant are shown (n = 5; *, $p < 0.05$).



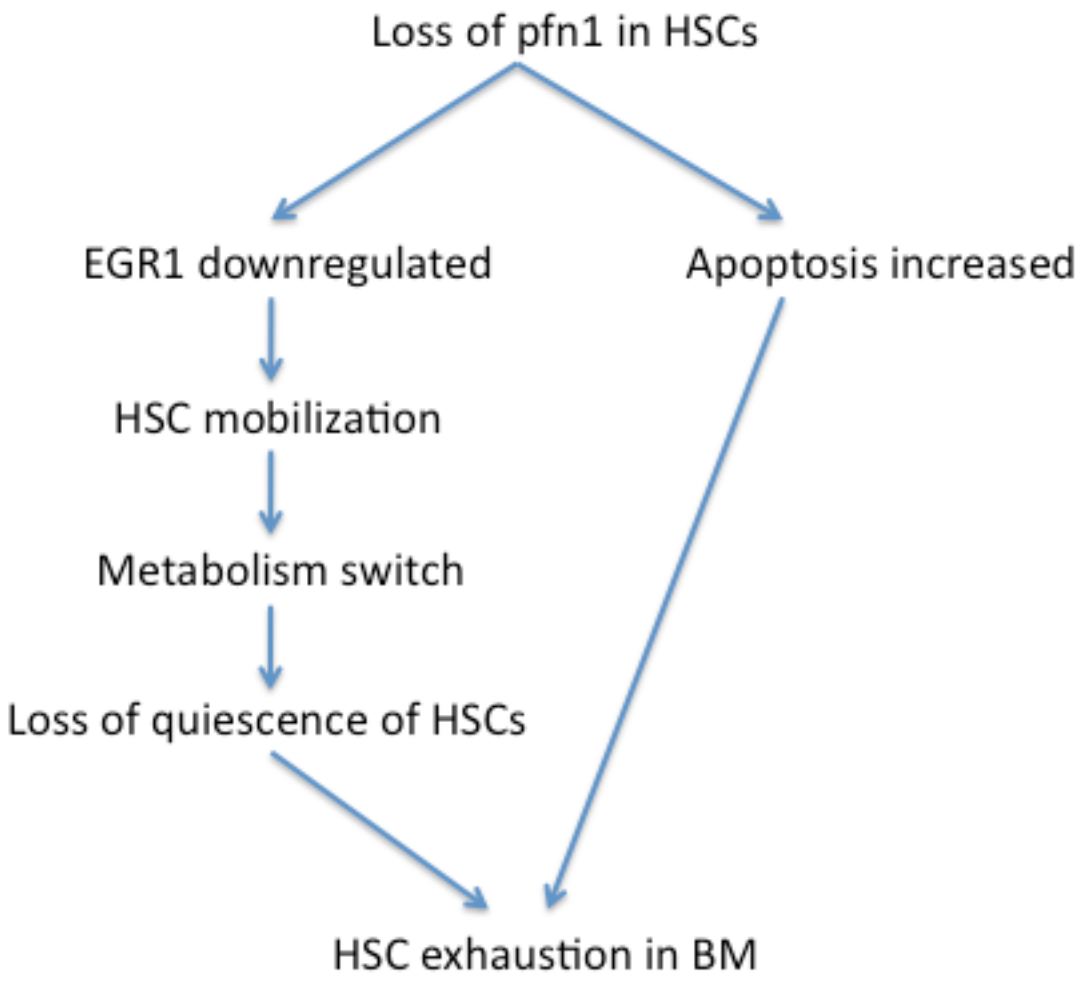
sFig. 3. EGR1 is downregulated in Sclpfn1 HSCs. (A) LT-HCSs from control or Sclpfn1 mice were isolated for measurement of EGR1 and MLL by using quantitative Real-time RT-PCR. (n = 3; *, $p < 0.05$). (B) Flow cytometry analysis of surface molecules on control and Sclpfn1 HSCs. (C) Lysates of Gα13 overexpressed 293T cells were co-immunoprecipitated with anti-PFN antibody or control goat IgG, and precipitation was then determined by western blotting using anti-Gα13 and anti-pfn1 antibodies. (D) G0 and G1 cells of LT-HSCs from the BM of control or EGR1 rescued mice were sorted for qRT-PCR analysis with the specific primers of Kreb cycle related genes.



sFig. 4. *Pfn1*$^{-/-}$ HSCs have normal differentiation potential and unaltered metabolic profiling *in vitro*. (A) The percentages of Mac-1^{+}/Gr-1^{+} myeloid cells in control and Sclpfn1 HSCs after 8-10 days in culture (n = 5; *, $p < 0.05$). (B-C) ATP production and oxygen consumption of control and Sclpfn1 HSCs were measured in culture.


A
Relative mRNA expression (/Control)
Control
Sclpfn1
PFKL
LDH
GLUT1
B
Gene expression in G1 relative to G0
Gene expression in Sclpfn1 relative to Control
β ACT
PDHA1
PDHA2
PDHB
Dbt(E2)
Dld(E3)
G0
G1
C
ATP (RLU)
Lin-
LK
LS
D
Oxygen consumption (RLU)
E
nM 13C-Lactate uM ATP
F
Control
Sclpfn1
0.00
87.20
12.80±1.63%
79.40
20.60±2.52%
49.49
50.51±4.63%
29.81
70.19±5.63%
FL4-H:: FL4-Height
FL2-H:: FL2-Height
HSC-G0
HSC-G1
TMRE
G
Sclpfn1+NAC
Pyronin Y
G1:26.4%
SG2M:5.7%
G0:63.5%
G1:39.5%
SG2M:5.5%
G0:48.9%
G1:20.8%
SG2M:9.6%
G0:61.4%
Hoechst
H
Kit
Sca-1
CD34
0.022%
0.012%
0.013%
Flk2
I
Gated on LSK cells
Annexin V
4.4%
10.6%
11.8%
CD34

sFig. 5. Metabolic switch of Sclpfn1 HSCs. (A) Comparison of mRNA levels of glycolysis related enzymes (PFKL, LDH and GLUT1) in control and Sclpfn1 HSCs. (B) Comparison of mRNA levels of Kreb cycle related enzymes (PDHA1, PDHA2, PDHB, Dbt(E2) and Dld(E3)) in G0 and G1 cells from control and Sclpfn1 HSCs. (C-E) Measurement of ATP content, oxygen consumption, and glycolytic flux in Lin^-, Lin^-Kit^+, and $Lin^-Sca\text{-}1^+$ cells) ($n = 3$; *, $p < 0.05$). (F) Mitochondrial potential in G0 and G1 cells from control and Sclpfn1 LT-HSCs were measured by TMRE staining (n=3). (G-I) Representative FACS plots of cell cycle status (G), HSCs (H), and Annexin V staining (I) of control, Sclpfn1, and Sclpfn1+NAC HSCs, for Fig. 5G-I.



sFig. 6. A working model of the pfn1 deletion resulted HSC exhaustion in BM. Deletion of pfn1 in HSCs leads to increased mobilization and metabolism switch, as well as apoptosis of HSCs, all of which contribute to bone marrow failure.

sTable 1. Primers for genotyping of WT and mutant mice

	Primer (5'-3')
Common	TGGAGCGGATCCAGCGAAGG
WT	GTCCCCAGCAGTCGGGACG
Mutant	GGACACCAACCTCAGCTGGC

These three PCR primers produced a 240, 340, and 700 bp band respectively for WT, flox, and null cells.

sTable 2. Primers for real-time RT-PCR

Primers	Forward (5'-3')	Reverse (5'-3')
EGR1	GAGCGAACAACCCTATGAGC	TGGGATAACTCGTCTCCACC
HIF1α	CGGCGAGAACGAGAAGAA	AAACTTCAGACTCTTTGCTTCG
PFKL	CCCTGACAGCAGCATTCATA	CTACCGTGGACCTGGAGAAA
LDH1	GTGCCCAGTTCTGGGTTAAG	CTGGGTCCTGGGAGAACAT
GLUT1	GAGTGTGGTGGATGGGATG	AACACTGGTGTCATCAACGC
PDHA1	GAAATGTGACCTTCATCGGCT	TGATCCGCCTTTAGCTCCATC
PDHA2	CTGTCTCACGTATTTTCGGGAA	AGCCGGTACAGGTCACATTTC
PDHB	AGGAGGGAATTGAATGTGAGGT	ACTGGCTTCTATGGCTTCGAT
Dbt(E2)	AGACTGACCTGTGTTCGCTAT	GAGTGACGTGGCTGACTGTA
Dld(E3)	GAGCTGGAGTCGTGTGTACC	CCTATCACTGTCACGTCAGCC