Srebf1c preserves hematopoietic stem cell function and survival as a switch of mitochondrial metabolism

Yukai Lu,1,3 Zihao Zhang,1,3 Song Wang,1 Yan Qi,1 Fang Chen,1 Yang Xu,1 Mingqiang Shen,1 Mo Chen,1 Naicheng Chen,1 Lijing Yang,1 Shilei Chen,1 Fengchao Wang,1 Yongping Su,1 Mengjia Hu,1,2,* and Junping Wang1,*

1State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Gaotanyan Street 30, Chongqing 400038, China
2Chinese PLA Center for Disease Control and Prevention, No. 20 Dongda Street, Fengtai District, Beijing 100071, China
3These authors contributed equally
*Correspondence: weichen1111@yahoo.com (M.H.), wangjunp@yahoo.com (J.W.)

https://doi.org/10.1016/j.stemcr.2022.01.011

SUMMARY

Mitochondria are fundamental but complex determinants for hematopoietic stem cell (HSC) maintenance. However, the factors involved in the regulation of mitochondrial metabolism in HSCs and the underlying mechanisms have not been fully elucidated. Here, we identify sterol regulatory element binding factor-1c (Srebf1c) as a key factor in maintaining HSC biology under both steady-state and stress conditions. Srebf1c knockout (Srebf1c−/−) mice display increased phenotypic HSCs and less HSC quiescence. In addition, Srebf1c deletion compromises the function and survival of HSCs in competitive transplantation or following chemotherapy and irradiation. Mechanistically, SREBF1c restrains the excessive activation of mammalian target of rapamycin (mTOR) signaling and mitochondrial metabolism in HSCs by regulating the expression of tuberous sclerosis complex 1 (Tsc1). Our study demonstrates that Srebf1c plays an important role in regulating HSC fate via the TSC1-mTOR-mitochondria axis.

INTRODUCTION

Hematopoietic stem cells (HSCs), which sit at the top of the hematopoietic hierarchy, can self-renew and differentiate into all kinds of blood cells over the lifespan (Carrelha et al., 2018; Pinho and Frenette, 2019). During the steady state, the majority of HSCs are retained in a specific bone marrow (BM) microenvironment and are maintained in a quiescent state (Cabez-Ballscheid et al., 2017; Mendelson and Frenette, 2014). It has been well established that the loss of quiescence may impair the long-term repopulation capability of HSCs, eventually resulting in the perturbation of hematopoietic homeostasis (Hou et al., 2015). Previous studies have identified many cell-intrinsic and cell-extrinsic factors that are involved in regulating HSC function (Pinho and Frenette, 2019). In recent years, increasing attention has been focused on cell metabolism, such as mitochondrial metabolism, fatty acid oxidation, glutamine metabolism, and amino acid catabolism, in adult stem cells (Khoa et al., 2020; Ludikhuize et al., 2020; Toyama et al., 2016; Villegas et al., 2019), but how metabolic changes affect the fate of HSCs remains incompletely understood.

In the hypoxic microenvironment, quiescent HSCs mainly use glycolysis to generate energy, which is required to preserve their self-renewal ability (Takubo et al., 2010, 2013). In response to various stress signals, anaerobic metabolism will switch to mitochondrial respiration to meet the energy demands for HSC proliferation (Wang et al., 2021).

Previous studies reported that the mammalian target of rapamycin (mTOR), AMP kinase (AMPK), peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α), and Ca2+ play a central role in mitochondrial energy metabolism (Cantó et al., 2009; Cunningham et al., 2007; Umemoto et al., 2018). These pathways mediate the partial role of other factors, such as TSC-1, TWIST1, LKB1, Dlk1-Gtl2, SRC-3, and MEK1, in regulating HSC biology (Baumgartner et al., 2018; Chen et al., 2008; Gan et al., 2008, 2010; Hu et al., 2018; Qian et al., 2016; Wang et al., 2021). Despite considerable study, the exquisite modulation of mitochondrial metabolism is still incompletely characterized.

Sterol regulatory element binding factor-1c (Srebf1c; also called SREBP1c/ADD1) is one of the highly conserved basic helix-loop-helix-leucine zipper family members. It has been shown that Srebf1c is an indispensable metabolic regulator in both humans and mice (Wang et al., 2015). As a transcription factor, SREBF1c can control the expression of key enzymes for fatty acid synthesis, such as fatty acid synthase (FAS), acetyl coenzyme A (CoA) carboxylase 1 (ACC1), and stearoyl-CoA desaturase 1 (SCD1), in adipocytes and liver cells (Wang et al., 2015). In addition, Srebf1c was reported to repress fatty acid metabolism and mitochondrial function in peripheral nerve cells by regulating the expression of peroxisome proliferator-activated receptor α (Pparα) ligands (Cermenate et al., 2015). Interestingly, another study showed that Srebf1c modulates glucose metabolism in natural killer (NK) cells (Asmann et al., 2017). These findings indicate that Srebf1c functions in a
cell context-dependent manner. However, it is still unclear whether Srebf1c can regulate HSC function by affecting specific metabolic processes.

To elucidate the role of Srebf1c in HSCs, we used a Srebf1c knockout (Srebf1c<sup>−/−</sup>) mouse model and found that loss of Srebf1c leads to significant proliferation and activation of phenotypic HSCs, accompanied by impairment in their hematopoietic reconstitution capacity. Mechanistically, SREBF1c inhibited the hyperactivation of the mTOR pathway and mitochondrial metabolism via transcriptional control of Tsc1 expression. Overall, our study provides new insight into the role of Srebf1c in maintaining HSC function and survival by modulating the TSC1-mTOR-mitochondria axis.

RESULTS

Srebf1c is enriched in HSCs, and its deficiency slightly affects mature hematopoiesis in mice

To understand the role of Srebf1c in hematopoiesis, we analyzed its expression pattern in the BM of mice. Quantitative real-time-PCR analysis showed that Srebf1c expression was relatively higher in hematopoietic stem and progenitor cells (HSPCs), especially in long-term HSCs (LT-HSCs), than in lineage-positive (Lin<sup>+</sup>) cells (Figure 1A). Then, a Srebf1c<sup>−/−</sup>/LacZ mouse model (Liang et al., 2002), which expresses the lacZ gene after Srebf1c deletion, was used in our study. Similar expression profiling was observed by flow cytometric analysis with fluorescent dye-β-D-galactopyranoside (FDG) staining (Figure 1B). These findings suggest that Srebf1c may play a distinctive role in HSC biology. By analyzing Srebf1c<sup>−/−</sup> and their littermate wild-type (WT) control mice, we found that the depletion of Srebf1c resulted in splenomegaly with increased B cell numbers (Figures 1C–1F and S1A–S1C). However, other conventional hematopoietic parameters were largely unchanged after Srebf1c knockout (Figures 1G–1I and S1D). Thus, these data indicate that Srebf1c deficiency has only a slight effect on mature hematopoiesis.

Srebf1c deletion disturbs normal HSPC pool

To further acquire insight into the role of Srebf1c in hematopoiesis, we conducted flow cytometric analysis on the HSPC compartment. Deletion of Srebf1c significantly increased the percentage and absolute number of Lin<sup>−</sup> Sca1<sup>+</sup> c-Kit<sup>+</sup> cells (LSKs), but not myeloid progenitors (MPs), in the BM (Figures 2A and 2B). We then examined the LSK subpopulation and observed that despite the unbalanced proportion of LT-HSCs, short-term HSCs (ST-HSCs), and multipotent progenitors (MPPs), the absolute number of all of the subpopulations was increased after Srebf1c deficiency (Figures 2C and 2D). Similar results were obtained using another combination of HSC markers, CD48 and CD150 (Figures 2E and 2F). In addition, Srebf1c<sup>−/−</sup> mice showed an increased number of megakaryocyte-erythroid progenitors (MEPs) but a decreased number of common myeloid progenitors (CMPs) in the BM (Figures 2G and 2H). In contrast, no significant alteration was observed in the number of granulocyte-macrophage progenitors (GMPs) or common lymphoid progenitors (CLPs) when Srebf1c was deleted (Figures 2G and 2H). Consistent with the HSC expansion in the BM, a marked increase in LSK number was found in the spleen and peripheral blood (PB) of Srebf1c-null mice (Figures 2I and S2). However, we observed increased numbers of MPs and CLPs in the spleens of Srebf1c<sup>−/−</sup> mice (Figure 2J). Thus, Srebf1c is required to sustain a normal HSPC pool in mice.

Srebf1c knockout leads to the loss of HSC quiescence

To determine whether Srebf1c deficiency affects HSC cell-cycle status, we performed Ki67 and Hoechst 33342 staining. Flow cytometric analysis revealed that the frequency of HSCs in G0 phase was reduced and the frequency in G1 and S/G2/M phases was increased in Srebf1c<sup>−/−</sup> mice (Figures 3A–3C and S3A). Consistently, the bromodeoxyuridine (BrDU) incorporation assay showed that Srebf1c deficiency promoted the proliferation of HSCs (Figures 3D and S3B). Next, quantitative real-time-PCR analysis showed that the expression of cell-cycle inhibitors, such as Cdkn1a and Cdkn1c, was downregulated, whereas the expression of Ccnb1, Ccne1, and Cdk2 was upregulated in Srebf1c<sup>−/−</sup> HSCs (Figure 3E). Proliferative active cells are more sensitive to genetic insults, such as ionizing radiation (IR) and chemotherapy (Sinha et al., 2019; Wang et al., 2016). As expected, Srebf1c<sup>−/−</sup> mice displayed reduced survival compared with WT mice following sequential 5-fluorouracil (5-FU) treatment (Figure 3F) or a lethal dose of total body irradiation (Figure 3G). Furthermore, annexin V and 7-aminoactinomycin D (7-AAD) staining showed an increased apoptosis rate in Srebf1c<sup>−/−</sup> HSCs (Figures 3H and S3C). Collectively, Srebf1c knockout drives the proliferation and accelerates the apoptosis of HSCs.

Srebf1c deficiency compromises the hematopoietic reconstitution ability of HSCs

To investigate whether deletion of Srebf1c affects HSC function, we conducted a homing assay and found that the homing efficiency was comparable between WT and Srebf1c<sup>−/−</sup> HSCs (Figure S4A). Then, we performed serial competitive BM transplantation (BMT) assays (Figure 4A). The percentages of Srebf1c<sup>−/−</sup> donor-derived cells were significantly reduced in recipients’ PB after primary and secondary BMT, accompanied by a defect in long-term
multilineage reconstitution (Figures 4B–4E). Consistently, Srebf1c−/− HSCs formed significantly fewer colonies in vitro than WT HSCs (Figure S4B). As Srebf1c was globally deleted in mice, we performed reciprocal BMT assays to assess how Srebf1c regulates HSC biology (intrinsic and/or extrinsic) (Figures 4F and 4I). Consistent with the phenotype of HSCs in Srebf1c-null mice, recipients transplanted with Srebf1c−/− BM cells showed increased HSC numbers and proliferation (Figures 4G, 4H, and S4C). In contrast, no significant difference in HSC phenotype was observed...
between WT and Srebf1c−/− mice that received CD45.1 BM cells (Figures 4J, 4K, and S4D). These results suggest that Srebf1c is required for maintaining HSC long-term repopulation possibly in a cell-intrinsic manner.

**Srebf1c ablation leads to significantly increased mitochondrial metabolism in HSCs**

To further explore the underlying mechanism by which Srebf1c regulates HSC quiescence and function, we conducted RNA sequencing (RNA-seq) using LSKs from WT and Srebf1c−/− mice. Bioinformatics analysis showed that 595 genes were upregulated and 1,542 genes were downregulated in Srebf1c−/− LSKs relative to WT controls (Figures 5A and 5B). Notably, the upregulated genes were significantly enriched in metabolic pathways (Figure 5C). Subsequently, we performed gene set enrichment analysis (GSEA) and found that proliferation-related HSC signatures were enriched in Srebf1c−/− HSCs, while quiescence-related signatures were enriched in WT HSCs (Figures 5D and S5A), consistent with the above findings. Although previous studies reported that Srebf1c regulates lipid synthesis (Wang et al., 2015), the expression of two key enzymes in this pathway was not significantly altered in HSCs following Srebf1c deficiency (Figure S5B). Interestingly, Srebf1c-deleted HSCs presented a significant upregulation of metabolic signatures, including the citrate cycle, carbon metabolism, oxidative phosphorylation, and mitochondrial organization compared to WT cells (Figure 5E). It has been shown that cell proliferation is always associated with a large cell size and active metabolism (Suda et al., 2011). HSCs from Srebf1c−/− mice showed a larger cell size (Figure S5C). Given that mitochondria play a central role in energy metabolism, which is closely associated with hematopoietic homeostasis (Liang et al., 2020; Mansell et al., 2021; Wang et al., 2021), we then measured the mitochondrial properties of HSCs. Mitochondrial mass, membrane potential, and the expression of mitochondria-related genes, such as Cyc1, Atp4a, Cox5a, Cox6a2, Ndufc1, and Ldh3a, were increased in Srebf1c-null HSCs (Figures 5F, 5G, and S5D–S5G). Notably, mitochondrial metabolism was comparable between WT and Srebf1c−/− MPs (Figures 5F and 5G), revealing that Srebf1c may play a unique role in HSCs (to a greater extent in LSKs). In addition, transmission electron microscopy (TEM) revealed that mitochondrial number and folds of cristae were increased in Srebf1c−/− HSCs (Figure 5H). Furthermore, we observed increased glucose uptake, oxygen consumption rate (OCR), and pyruvate dehydrogenase (PDH) activity in Srebf1c−/− HSCs (Figures 5I, 5J, and 5K), accompanied by elevated ATP and reactive oxygen species (ROS) levels (Figures 5I and 5J). In contrast, decreased extracellular acidification rate (ECAR), lactate dehydrogenase (LDH) activity, and pyruvate and lactate levels were found in Srebf1c−/− HSCs (Figures 5J, 5M, S5H, and S5I). Overall, these data suggest that Srebf1c maintains HSC function by suppressing mitochondrial activity.

**SREBF1c inhibits the hyperactivation of mTOR signaling via the transcriptional control of Tsc1**

mTOR signaling plays a critical role in regulating mitochondrial metabolism and HSC maintenance (Liu et al., 2019; Qian et al., 2016). Notably, a markedly enriched mTOR complex 1 (mTORC1) signaling in Srebf1c−/− HSCs was observed in our GSEA data (Figure 6A). To further verify whether the mTOR pathway is activated in Srebf1c−/− HSCs, we examined the key points of this pathway. As anticipated, the phosphorylation levels of mTOR, S6, and 4E-BP1 and the expression of PGC-1α, a master regulator of mitochondrial metabolism, were significantly increased in HSCs after Srebf1c deletion (Figures 6B–6D and S6A). Akt is an upstream activator of mTOR, but the level of p-Akt was not altered in HSCs in the absence of Srebf1c (Figure S6B).

---

**Figure 2. Srebf1c deletion disturbs normal HSPC pool**

(A) Representative flow cytometric plots of the percentage of HSPCs in WT and Srebf1c−/− BM. (B) The percentage (left) and absolute number (2 femurs and tibias; right) of MPs and LSKs in the BM from WT and Srebf1c−/− mice (n = 5). (C) Representative flow cytometric plots of the percentage of HSC subpopulations in WT and Srebf1c−/− LSKs. (D) The percentage of subpopulations in LSKs (left) and the absolute number (2 femurs and tibias) of LT-HSCs, ST-HSCs, and MPPs in the BM (right) of WT and Srebf1c−/− mice (n = 5). (E) Representative flow cytometric plots of the percentage of signaling lymphocyte activation molecule (SLAM)-HSCs (CD150+CD48− LSKs) in WT and Srebf1c−/− LSKs. (F) The percentage of SLAM-HSCs in LSKs (left) and the absolute number (2 femurs and tibias) of SLAM-HSCs in the BM (right) of WT and Srebf1c−/− mice (n = 5). (G) Representative flow cytometric plots of the percentage of GMPs, MEPs, CMPs (left), and CLPs (right) in the BM of WT and Srebf1c−/− mice. (H) The absolute number (2 femurs and tibias) of GMPs, MEPs, CMPs, and CLPs from WT and Srebf1c−/− BM (n = 6). (I) Representative flow cytometric plots of the percentage of splenic LSKs (left) and the absolute number of LSKs in the spleens (right) of WT and Srebf1c−/− mice (n = 6). (J) The absolute number of GMPs, MEPs, CMPs, and CLPs in the spleens of WT and Srebf1c−/− mice (n = 6).

**Please cite this article in press as:** Lu et al., Srebf1c preserves hematopoietic stem cell function and survival as a switch of mitochondrial metabolism, Stem Cell Reports (2022), https://doi.org/10.1016/j.stemcr.2022.01.011
Please cite this article in press as: Lu et al., Srebf1c preserves hematopoietic stem cell function and survival as a switch of mitochondrial metabolism, Stem Cell Reports (2022), https://doi.org/10.1016/j.stemcr.2022.01.011
However, studies have shown that mTOR signaling is negatively regulated by tuberous sclerosis proteins 1 and 2 (TSC1/2) (Hay, 2005; Lee et al., 2007). In fact, the mRNA and protein levels of TSC1, but not TSC2, were lower in Srebf1c<sup>−/−</sup> HSCs than in control cells (Figures 6E–6G and S6C–S6E). Moreover, we noticed that Srebf1c<sup>−/−</sup> mice exhibited HSC phenotypes similar to those of Tsc1<sup>−/−</sup> mice (Chen et al., 2008; Gan et al., 2008). Therefore, we speculated that SREBF1c restricts mTOR activity and mitochondrial metabolism in HSCs by modulating TSC1 expression (Figure 6H). Subsequently, bioinformatic prediction revealed that there was a potential binding site of SREBF1c in the Tsc1 promoter region (Figure 6I), which was further confirmed by chromatin immunoprecipitation (ChIP) assays (Figure 6J). These results demonstrate that SREBF1c suppresses mTOR signaling and downstream mitochondrial metabolism by directly controlling the transcription of Tsc1.

Inhibition of mTOR activation or scavenging ROS improves HSC defects in Srebf1c-deleted mice

To further determine whether hyperactivation of the mTOR pathway is responsible for HSC defects, Srebflc<sup>−/−</sup> mice were administered the mTORC1 inhibitor rapamycin. We found that rapamycin treatment significantly inhibited mTOR activity, mitochondrial mass, mitochondrial membrane potential, glucose uptake, and ROS production in Srebf1c-null HSCs (Figures 7A–7G and S7A). In addition, phenotypic expansion and aberrant proliferation of HSCs in Srebf1c<sup>−/−</sup> mice were largely reversed after rapamycin treatment (Figures 7H, 7I, and S7B). Inhibition of mTOR activity by rapamycin significantly rescued the impaired function of Srebf1c<sup>−/−</sup> HSCs (Figures 7J and S7C). Finally, to confirm whether the elevated ROS levels caused by mitochondrial activation contribute to HSC defects in Srebf1c<sup>−/−</sup> mice, the effective ROS scavenger N-acetyl-l-cysteine (NAC) was applied (Figure S7D). We found that NAC treatment partially reversed the phenotypic and functional defects of Srebflc-deficient HSCs (Figures S7E–S7I). Our data demonstrate that Srebf1c maintains HSC homeostasis via the limitation of mTOR activation and mitochondrial metabolism (Figure 7K).

**DISCUSSION**

HSC homeostasis needs to be exquisitely regulated for the replenishment of all blood cells under both steady-state and stress conditions (Mendelson and Frenette, 2014; Pinho and Frenette, 2019). It has been reported that transcription factors, epigenetic factors, multiple RNAs (micro-RNAs, long-length noncoding RNAs, circular RNAs), and cytokines participate in the modulation of HSC biology (Hou et al., 2015; Hu et al., 2021; Li et al., 2018; Qian et al., 2016; Xia et al., 2018; Yamashita and Passegue, 2019). Notably, energy metabolism is emerging as a regulatory element of HSC maintenance, whereas the underlying mechanism remains elusive. Here, we reported for the first time that SREBF1c inhibits the mTOR pathway and downstream mitochondrial metabolism by regulating the transcription of Tsc1, which is required for promoting HSC maintenance in mice.

The Srebfs family, including Srebfla, Srebflc, and Srebfl2 isoforms, displays incompletely overlapping functions for regulating several metabolic processes (Wang et al., 2015). For example, Srebfl2 and Srebfla can participate in cholesterol biosynthesis, while Srebflc preferentially governs lipogenesis (Liang et al., 2002). Cholesterol-induced Srebfl2 activation promotes HSPC expansion in atherosclerotic cardiovascular disease in zebrafish (Gu et al., 2019). However, less attention has been focused on the role of Srebflc in hematopoiesis. In the present study, we showed that Srebflc is relatively enriched in HSCs in the hematopoietic system, suggesting that Srebflc may participate in the regulation of HSC behavior. Using a Srebflc<sup>−/−</sup> mouse model, we found that Srebflc deficiency leads to an expansion of

**Figure 3.** Srebflc knockout leads to the loss of HSC quiescence (A) Representative flow cytometric plots of cell-cycle distribution of LSKs and LT-HSCs from WT and Srebflc<sup>−/−</sup> mice by Ki67/Hoechst 33342 staining. (B) The frequency of LSKs (left) and LT-HSCs (right) in G0, G1, and S/G2/M phases of WT and Srebflc<sup>−/−</sup> mice (n = 5). (C) Flow cytometric analysis of cell-cycle distribution of SLAM-HSCs from WT and Srebflc<sup>−/−</sup> mice (n = 5). (D) Representative flow cytometric plots of cell proliferation with BrdU staining (left) and the proportion of BrdU<sup>+</sup> cells (right) in LSKs and LT-HSCs of WT and Srebflc<sup>−/−</sup> mice (n = 5). (E) The relative expression levels of cell-cycle-related genes in LT-HSCs sorted from WT and Srebflc<sup>−/−</sup> mice were measured by quantitative real-time-PCR (n = 3). (F and G) Kaplan-Meier survival analysis of WT and Srebflc<sup>−/−</sup> mice following (F) sequential 5-FU administration and (G) 7.5 Gy irradiation (n = 9–10). (H) Representative flow cytometric plots of apoptosis with annexin V/7-AAD staining (left) and the percentage of annexin V<sup>+</sup> 7-AAD<sup>−</sup> cells (right) in LSKs and LT-HSCs from WT and Srebflc<sup>−/−</sup> mice (n = 5). **p < 0.01.
Figure 4. Loss of Srebf1c compromises the long-term reconstitution ability of HSCs

(A) Schematic for competitive transplantation with WT and Srebf1c<sup>−/−</sup> BM cells.

(B–E) Flow cytometric analysis of the donor percentages (B and D) and multilineage (T cells, B cells, myeloid cells) reconstitution (C and E) in the PB of CD45.1<sup>+</sup> recipient mice at 16 weeks after the first (B and C) and second (D and E) transplantations (n = 6–8).

(legend continued on next page)
phenotypic HSCs in the BM. In addition, increased HSPC and mature cell numbers in the spleen, accompanied by an enlarged spleen, were observed in Srebf1c−/− mice, implying that the loss of Srebf1c may promote extramedullary hematopoiesis. In the steady state, HSCs with robust self-renewal potency are in a quiescent state. This property can protect HSCs from stress-induced injury and prevent their premature aging and exhaustion during long-term hematopoietic output (Takubo et al., 2010; Wang et al., 2021). Previous studies have shown that Srebf1c maintains the quiescence of hepatic satellite cells, but promotes the proliferation of pancreatic β cells (Lee et al., 2019; Su et al., 2020). In our study, we observed that the deletion of Srebf1c significantly impaired HSC quiescence, resulting in increased sensitivity to 5-FU and irradiation and decreased long-term repopulation capacity. HSC biology is co-regulated by cell-intrinsic or -extrinsic regulators in the BM niche (Pinho and Frenette, 2019). In our study, Srebf1c is globally deleted in mice. Although the reciprocal BMT assays and the in vitro colony-forming assay suggest that the defective function of Srebf1c−/− HSCs is probably cell intrinsic, we could not rule out the possibility of engraftment defect, developmental effects, or delayed environmental effect. To bring a deeper understanding of mechanism, a conditional knockout mouse model would be better.

Our previous studies and others have indicated that mitochondria emerge as drivers of HSC fate (Hu et al., 2018; Wang et al., 2021). Active mitochondrial metabolism is not compatible with quiescent HSCs, which mainly rely on glycolysis for energy production (Takubo et al., 2013). It has been reported that Srebf1c is involved in multiple metabolic processes, including lipid synthesis, fatty acid catabolism, and glycolysis (Wang et al., 2015), whereas whether Srebf1c regulates energy metabolism in HSCs remains unknown. Here, we showed that Srebf1c deletion results in a markedly increased mitochondrial function in HSCs, accompanied by elevated ROS levels. However, HSC impairment may be uncoupled from ROS elevation caused by mitochondrial abnormalities in some circumstances (Filippi and Ghaffari, 2019). In our study, scavenging ROS by NAC treatment partially rescued the defects of Srebf1c-null HSCs. These results demonstrate that elevated ROS levels induced by mitochondrial activation may be an important factor for HSC impairment in Srebf1c−/− mice.

mTOR is a serine/threonine kinase that exists in two complexes, mTORC1 and mTORC2 (Saxton and Sabatini, 2017). It is known that mTORC1, sensitive to rapamycin, participates in cell growth, mRNA translation, and protein synthesis (Inoki et al., 2002). In contrast, mTORC2 is insensitive to rapamycin, regulating cell survival, cytoskeleton organization, and gluconeogenesis (Hagiwara et al., 2012; Jacinto et al., 2004). Specifically, mTORC1 also controls mitochondrial biogenesis and activity by 4E-BP-dependent translational regulation or activating PGC-1α (Cunningham et al., 2007; Morita et al., 2013). Interestingly, either inhibition or activation of mTORC1 impairs HSC quiescence and hematopoietic potential (Ghosh et al., 2016; Kalaitzidis et al., 2012), suggesting that mTORC1 activity should be exquisitely regulated. Here, our findings revealed that the loss of Srebf1c leads to the hyperactivation of mTORC1 signaling and increased downstream protein levels in HSCs. As a result, rapamycin treatment largely corrected the increased mitochondrial activity and rescued the defective quiescence and function of Srebf1c−/− HSCs, suggesting that the hyperactive mTORC1 and mitochondrial metabolism may occur earlier than HSC proliferation and impairment. Overall, we demonstrate that Srebf1c maintains HSC homeostasis mainly by limiting the activity of mTORC1 signaling.

Researchers have reported that Akt plays a central role in the activation of the mTORC1 pathway (Hay, 2005; Qian et al., 2016). However, no significant difference in p-Akt levels was observed between WT and Srebf1c−/− HSCs, suggesting that Srebf1c deficiency-induced mTORC1 activation is not mediated by increased Akt activity. In addition, TSC1 and TSC2 are recognized as suppressing mTORC1 activity in many tissues (Hay, 2005; Lee et al., 2007). Previous studies reported that Tsc1 deletion increases HSC proliferation due to the abnormal activation of the mTORC1 pathway, leading to short-term expansion but loss of long-term hematopoietic function (Chen et al., 2008; Gan et al., 2008). Interestingly, the phenotypes of HSCs were comparable between Srebf1c−/− and Tsc1−/− mice. Both the mRNA and protein levels of TSC1 were significantly reduced after Srebf1c deletion. Furthermore, ChIP assays revealed that SREBF1c controls the transcription of Tsc1 by directly binding to its promoter region. Although TSC1 and TSC2 are homologous proteins, little is known about the role of TSC2 in HSCs. Moreover, our study showed that the expression of TSC2 was not altered in HSCs following Srebf1c ablation. Srebf1c is well known to regulate the expression of many lipid metabolism-related target genes, such as Fasn and Acc1 (Wang et al., 2015).
Please cite this article in press as: Lu et al., *Srebff1c* preserves hematopoietic stem cell function and survival as a switch of mitochondrial metabolism, Stem Cell Reports (2022), https://doi.org/10.1016/j.stemcr.2022.01.011

---

**A**  
*Srebff1c* KO vs WT  
595  
1542  
Fold change >2.0  
*p*<0.05

**B**  
Up Gene Pathway Enrichment  
- Metabolic pathways  
- Transcriptional misregulation in cancer  
- ITPC and Phagosome  
- Viral protein interaction with cytokine  
- Systemic lupus erythematosus  
- Cytokine-cytokine receptor interaction  
- Tuberculosis  
- Acute myeloid leukemia  
- Toll-like receptor signaling pathway  
- Biosynthesis of amino acids

**D**  
Proliferative HSC Signature  
NES=2.96  
*p*=0.000  
FDR q<0.000  
Mobilized HSC Signature  
NES=2.62  
*p*=0.000  
FDR q<0.000  
HSC Signature  
NES=2.50  
*p*=0.000  
FDR q<0.000  
Quiescent HSC Signature  
NES=2.04  
*p*=0.000  
FDR q<0.000

**E**  
Citrate Cycle  
NES=2.01  
*p*=0.000  
FDR q<0.002  
Carbon Metabolism  
NES=1.88  
*p*=0.000  
FDR q<0.005  
Oxidative Phosphorylation  
NES=2.47  
*p*=0.000  
FDR q<0.002  
Mitochondrial Organization  
NES=1.68  
*p*=0.000  
FDR q<0.000

**F**  
LT-HSC  
MTG  
WT  
*Srebff1c*<sup>−/−</sup>

**G**  
LT-HSC  
MTG  
WT  
*Srebff1c*<sup>−/−</sup>

**H**  
WT  
*Srebff1c*<sup>−/−</sup>

**I**  
OCR (nmol/min)  
Glucose

**J**  
ECAR (mPm/min)  
Glucose

**K**  
Relative ATP level  
Lin<sup>+</sup>  
LT-HSC

**L**  
Count

**M**  
DCFH-DA  
Relative lactate level  
Lin<sup>+</sup>  
LT-HSC

*legend on next page*
However, we did not observe any changes in Fasn and Acc1 expression in Srebf1c−/− HSCs. However, previous studies reported that SREBF1c acts downstream of mTORC1 to facilitate lipid biosynthesis in liver and Schwann cells (Normen et al., 2014; Yecies et al., 2011). Combined with these findings, we reasonably speculate that there may be a negative feedback loop between SREBF1c and mTORC1, which controls the metabolic balance in HSCs.

In conclusion, our study demonstrates that Srebf1c plays an important role in orchestrating HSC fate via restriction of the hyperactivation of mTORC1 activity and mitochondrial metabolism by regulating Tsc1 transcription. These findings improve our understanding of HSC biology and will give rise to novel avenues to facilitate HSC maintenance.

**EXPERIMENTAL PROCEDURES**

**Mice**

C57BL/6J mice were purchased from the Institute of Zoology (Chinese Academy of Sciences, Beijing, China). Srebf1c−/− mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA), and littermate WT mice served as controls. B6 SJL mice (CD45.1) were a kind gift from Prof. Jinyong Wang (Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Science, Guangzhou, China). All of the mice used were 8–10 weeks old. The animal experiments were performed according to the experimental procedures approved by the Animal Care Committee of the Third Military Medical University (Chongqing, China).

**Flow cytometric analysis and sorting**

Single-cell suspensions of mouse BM, spleen, and PB samples were prepared as we described previously (Hu et al., 2018, 2021). For hematopoietic cell phenotype analysis, cells were stained with antibodies identifying the following surface markers: Sca-1, c-Kit, CD34, Flk2, CD150, CD48, CD127, CD16/32, Gr-1, Mac-1, B220, CD3e, CD45.1, CD45.2, and the lineage cocktail (CD3e, Mac-1, Gr-1, B220, and Ter-119). Apoptosis, cell cycle, BrdU incorporation assay, and intracellular staining were performed as we described previously (Hu et al., 2018, 2021). All monoclonal antibodies (mAbs) were purchased from eBioscience (San Diego, CA, USA) or BioLegend (San Diego, CA, USA). Samples were detected by flow cytometry using a FACSVersa (BD Biosciences, San Jose, CA, USA) or FACSFortessa (BD Biosciences) and the data were analyzed using FlowJo 10.0 software (TreeStar, San Carlos, CA, USA).

For HSPC sorting, BM cells were first enriched by a Direct Lineage Cell Depletion Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer’s instructions. Then, enriched Lin− cells were stained with the above surface markers, 4',6-Diamidino-2-phenylindole (DAPI; Sigma, St. Louis, MO, USA) and trypan blue (Thermo Fisher Scientific, Grand Island, NY, USA) were used to confirm cell viability. Samples were sorted by a FACSAria II or FACSAria III sorter (BD Biosciences). Further details of flow cytometric antibodies are provided in Table S1.

**Transplantation assays**

The homing assay was performed as previously described (Gu et al., 2016). Briefly, 2 × 10⁶ LSKs isolated from WT or Srebf1c−/− mice (CD45.2) were transplanted into lethally irradiated (10 Gy) CD45.1+ mice. After 16 h, the homing efficiency was analyzed by flow cytometry. For competitive transplantation assays, 1 × 10⁶ BM cells from WT or Srebf1c−/− mice (CD45.2) along with an equivalent number of CD45.1+ BM cells were injected intravenously into recipient mice (CD45.1) after lethal irradiation (10 Gy). At 16 weeks after transplantation, the recipient mice (CD45.1/CD45.2) from the first transplantation were euthanized and then 1 × 10⁶ BM cells were transplanted into new lethally irradiated (10 Gy) recipients (CD45.1). The chimerism level in the PB of the recipient mice was measured at the indicated time after transplantation. For reciprocal transplantation assays, 1 × 10⁶ BM cells from WT or Srebf1c−/− mice (CD45.2) were transplanted into lethally irradiated (10 Gy) recipients (CD45.1), and 1 × 10⁶ BM cells from CD45.1 mice were transplanted into lethally irradiated (10 Gy) WT or Srebf1c−/− recipients (CD45.2). At 16 weeks after transplantation, HSC phenotypes in the recipient mice were detected by flow cytometry.
Figure 6. SREBF1c inhibits the hyperactivation of mTOR signaling via the transcriptional control of Tsc1

(A) GSEA of mTORC1 pathway in RNA-seq data from WT and Srebf1c<sup>−/−</sup> LSKs.

(B–D) Flow cytometric analysis of the expressions of (B) p-mTOR (S2448), (C) p-4E-BP1 (T37/46), and (D) PGC-1α in MPs, LSKs, and LT-HSCs of WT and Srebf1c<sup>−/−</sup> mice (n = 5).

(E) Quantitative real-time-PCR analysis of Tsc1 expression in WT and Srebf1c<sup>−/−</sup> LT-HSCs (n = 3).

(F) Western blot analysis of TSC1 protein expression in LSKs sorted from WT and Srebf1c<sup>−/−</sup> mice. Representative Western blot of 3 biological replicates is shown at left and quantitative analysis of the relative TSC1:glyceraldehyde 3-phosphate dehydrogenase (GAPDH) ratio is shown at right.

(G) Flow cytometric analysis of the expression of TSC1 in MPs, LSKs, and LT-HSCs from WT and Srebf1c<sup>−/−</sup> mice (n = 5).

(H) Immunofluorescence staining of TSC1 (green) and TOMM20 (red) in the LT-HSCs sorted from WT and Srebf1c<sup>−/−</sup> mice. Nuclei were stained with DAPI (blue). The scale bar represents 5 μm.

(legend continued on next page)
Mitochondrial mass, mitochondrial membrane potential, ROS levels, and glucose uptake analysis
WT and Srebfi1c−/− cells were first incubated with HSPC markers. Then, samples were stained with 20 nM Mito Tracker Green (MTG; Thermo Fisher), 20 nM tetramethylrhodamine methyl ester (TMRM; Thermo Fisher), 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; Beyotime, Shanghai, China) or 20 μM 2′-deoxy-2-(7-nitro-2,1,3-benzoxadiazol-4-yl) amino (2-NBDG; Thermo Fisher) as previously described (Hu et al., 2018; Mansell et al., 2021).

ATP measurement
ATP measurement was performed as previously described, with minor modifications (Mansell et al., 2021). In brief, 1 × 10⁶ LT-HSCs or Lin− cells sorted from WT and Srebfi1c−/− mice were resuspended in 100 μL PBS containing 2% fetal bovine serum (FBS), and then transferred into a 96-well plate, followed by the addition of 50 μL detergent and substrate solution. Finally, the ATP level was measured using the Luminescent ATP Detection Assay Kit (Abcam, Cambridge, UK).

Seahorse assays
The OCR and extracellular acidification rate were detected using the Agilent Seahorse XFp Cell Mito Stress Test Kit or the Glycolysis Stress Test Kit (Agilent Technologies, Santa Clara, CA, USA) as previously described (Hu et al., 2018; Rao et al., 2019). Briefly, sorted LSKs (5 × 10⁴) were plated into miniplates precoated with Cell-Tak (BD Biosciences). For the Mito Stress Test, cells were suspended in XF assay medium containing 1 mM pyruvate, 10 mM glucose, and 2 mM glutamine (pH 7.4), and then incubated in a CO₂-free incubator at 37°C. Finally, respiration was measured by an Agilent Seahorse XP analyzer (Agilent Technologies) after sequential addition of 1 μM oligomycin, 1 μM carbonic anhydrase p-triflouroacetiloxypyroxenylhydrozine (FCCP), and 0.5 μM rotenone/antimycin A (Agilent Technologies). For the glycolysis stress test, cells were suspended in XF base medium supplemented with 1 mM glutamine (pH 7.4) and then incubated in a CO₂-free incubator at 37°C. Finally, the glycolysis stress test was measured by an Agilent Seahorse XP analyzer after the sequential addition of 10 mM glucose, 2 μM oligomycin, and 50 mM 2-deoxy-α-glucose (2-DG) (Agilent Technologies).

RNA-seq
Transcriptome sequencing was conducted at Shanghai Sinomics (Shanghai, China). In brief, LSKs (1 × 10⁵ per sample) were sorted from WT and Srebfi1c−/− mice by flow cytometry, and total RNA was extracted using the RNAqueous Kit (Ambion, Darmstadt, Germany). Paired-end libraries were synthesized by using the TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA, USA) following the user manuals. After purification and enrichment, library fragments were loaded into cBot to generate clusters, followed by sequencing on an Illumina NovaSeq 6000. Differentially expressed genes were defined by fold change >2.0 and p < 0.05, and then used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. GSEA was performed using GSEA_4.0.3 software, and gene sets were summarized from previous studies (Cabezas-Wallscheid et al., 2017; Nakagawa and Rathinam, 2018). The sequencing data were submitted to the BioProject database: PRJNA723365.

Statistical analysis
Data analysis was performed using Graphpad Prism 6.0 software (La Jolla, CA, USA). All of the experiments were independently performed at least three times. Unpaired Student’s t test (two-tailed) was used for two group comparisons, and one-way ANOVA was used for multiple group comparisons. The log rank test and Kaplan-Meier curve were used for survival analysis. All of the data were shown as the mean ± SD. *p < 0.05 and **p < 0.01 are depicted as statistically significant differences.

Data and code availability
The data that support the findings of this study are available from the BioProject database: PRJNA723365.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.stemcr.2022.01.011.

AUTHOR CONTRIBUTIONS
Y.L. and Z.Z. designed the study, performed the experiments, analyzed the data, and wrote the paper. S.W., Y.Q., and F.C. performed some animal experiments and analyzed the data. Y.X., M.S., and M.C. participated in some in vitro experiments. N.C., L.Y., and S.C. participated in the data analysis. E.W. and Y.S. participated in the initial experimental design and discussed the manuscript. M.H. and J.W. conceived and supervised the study and revised the manuscript.

CONFLICTS OF INTEREST
The authors declare no competing interests.

ACKNOWLEDGMENTS
We thank Prof. Jinyong Wang for the gift of the CD45.1 mice, Yang Liu for technical support in flow cytometry, and Liting Wang for technical support in immunofluorescence microscopy. This work was supported by grants from the National Natural Science Foundation of China (nos. 81930090 and 81725019) and the Scientific Research Project of PLA (AWS16)014).

(I) Schematic diagram of the predicted SREBF1c binding site in the Tsc1 promoter region.
(J) ChIP-PCR analysis of the binding of SREBF1c to the promoter region of Tsc1 in WT and Srebfi1c−/− LSKs. Immunoglobulin G (IgG) served as a negative control.
**p < 0.01.
Figure 7. Inhibition of mTOR activation improves HSC defects in Srebf1c-deleted mice

(A–I) WT and Srebf1c−/− mice were administered intraperitoneally (i.p.) vehicle or rapamycin (4 mg/kg) every other day for 2 weeks. Mean fluorescence intensities (MFIs) of (A) p-mTOR (S2448), (B) p-S6 (Ser235, Ser236), (C) p-4E-BP1 (T37/46), (D) PGC-1α, (E) MTG, (F) TMRM, and (G) DCFH-DA in LT-HSCs from WT and Srebf1c−/− mice were analyzed by flow cytometry (n = 5). (H) The absolute number (2 femurs and tibias) and (I) cell-cycle distribution of LT-HSCs from WT and Srebf1c−/− mice were analyzed by flow cytometry (n = 5).

(legend continued on next page)
lethally irradiated CD45.1+ recipients. One week after transplantation, recipients received vehicle or rapamycin (4 mg/kg) by i.p. injection.

**p < 0.01.

**Schematic overview of the proposed model demonstrating the role of Srebf1c in regulating HSC homeostasis.

REFERENCES

Assmann, N., O’Brien, K.L., Donnelly, R.P., Dyck, L., Zaitz-Bittencourt, V., Loftus, R.M., Heinrich, P., Oefner, P.J., Lynch, L., Gardiner, C.M., et al. (2017). Srebp-controlled glucose metabolism is essential for NK cell functional responses. Nat. Immunol. 18, 1197–1206. https://doi.org/10.1038/ni.3838.

Baumgartner, C., Toifl, S., Farlik, M., Halbritter, F., Scheicher, R., Fischer, I., Sexl, V., Bock, C., and Baccarini, M. (2018). An ERK-dependent feedback mechanism prevents hematopoietic stem cell exhaustion. Cell Stem Cell 22, 879–892.e6. https://doi.org/10.1016/j.stem.2018.05.003.

Cabezas-Wallscheid, N., Buettner, F., Sommerkamp, P., Klimmek, D., Ladel, L., Thalheimer, F.B., Pastor-Flores, D., Roma, L.P., Rendler, S., Zeisberger, P., et al. (2017). Vitamin A-retinoic acid signaling regulates hematopoietic stem cell dormancy. Cell 169, 807–823.e19. https://doi.org/10.1016/j.cell.2017.04.018.

Cantó, C., Gerhart-Hines, Z., Feige, J.N., Lagouge, M., Noriega, L., Milne, J.C., Elliott, P.J., Puigserver, P., and Auwerx, J. (2009). AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 458, 1056–1060. https://doi.org/10.1038/nature07813.

Carrelha, J., Meng, Y., Kettyle, L.M., Luis, T.C., Norfo, R., Alcolea, V., Boukarabila, H., Grasso, F., Gambardella, A., Grover, A., et al. (2018). Hierarchically related lineage-restricted fates of multipotent haematopoietic stem cells. Nature 554, 106–111. https://doi.org/10.1038/s41586-018-0037-z.

Cermenati, G., Audano, M., Giatti, S., Carozzi, V., Porretta-Serapiglia, C., Pettinato, E., Ferri, C., D’Antonio, M., De Fabiani, E., Crestani, M., et al. (2015). Lack of sterol regulatory element binding factor-1c imposes glial fatty acid utilization leading to peripheral neuropathy. Cell Metab. 21, 571–583. https://doi.org/10.1016/j.cmet.2015.02.016.

Chen, C., Liu, Y., Liu, R., Ikenoue, T., Guan, K.L., Liu, Y., and Zheng, P. (2008). TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J. Exp. Med. 205, 2397–2408. https://doi.org/10.1084/jem.20081297.

Cunningham, J.T., Rodgers, J.T., Arlow, D.H., Vazquez, F., Mootha, V.K., and Puigserver, P. (2007). mTOR controls mitochondrial oxidative function through a YY1–PGC-1α transcriptional complex. Nature 450, 736–740. https://doi.org/10.1038/nature06322.

Filippi, M.D., and Ghaffari, S. (2019). Mitochondria in the maintenance of hematopoietic stem cells: new perspectives and opportunities. Blood 133, 1943–1952. https://doi.org/10.1182/blood-2018-10-808873.

Gan, B., Hu, J., Jiang, S., Liu, Y., Sahin, E., Zhuang, L., Fletcher-Sanokan, E., Colla, S., Wang, Y.A., Chin, L., and Depinho, R.A. (2010). Lkb1 regulates quiescence and metabolic homeostasis of hematopoietic stem cells. Nature 468, 701–704. https://doi.org/10.1038/nature09595.

Gan, B., Sahin, E., Jiang, S., Sanchez-Aguilera, A., Scott, K.L., Chin, L., Williams, D.A., Kwiatkowski, D.J., and Depinho, R.A. (2008). mTORC1-dependent and -independent regulation of stem cell renewal, differentiation, and mobilization. Proc. Natl. Acad. Sci. U S A 105, 19384–19389. https://doi.org/10.1073/pnas.0810584105.

Ghosh, J., Kobayashi, M., Ramdas, B., Chatterjee, A., Ma, P., Mali, R.S., Carlsson, N., Liu, Y., Plas, D.R., Chan, R.J., and Kapur, R. (2016). S6K1 regulates hematopoietic stem cell self-renewal and leukemia maintenance. J. Clin. Invest. 126, 2621–2625. https://doi.org/10.1172/JCI84565.

Gu, Q., Yang, X., Lv, J., Zhang, J., Xia, B., Kim, J.D., Wang, R., Xiong, F., Meng, S., Clements, T.P., et al. (2019). AIBP-mediated cholesterol efflux instructs hematopoietic stem and progenitor cell fate. Science 363, 1085–1088. https://doi.org/10.1126/science.aav1749.

Gu, Y., Jones, A.E., Yang, W., Liu, S., Dai, Q., Liu, Y., Swindle, C.S., Zhou, D., Zhang, Z., Ryan, T.M., et al. (2016). The histone H2A deubiquitinase Usp16 regulates hematopoiesis and hematopoietic stem cell function. Proc. Natl. Acad. Sci. U S A 113, E51–E60. https://doi.org/10.1073/pnas.1517041113.

Hagiwara, A., Cornu, M., Cybulski, N., Polak, P., Betz, C., Trapani, F., Terracciano, L., Heim, M.H., Ruegg, M.A., and Hall, M.N. (2012). Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c. Cell Metab. 15, 725–738. https://doi.org/10.1016/j.cmet.2012.03.015.

Hay, N. (2005). The Akt-mTOR tango and its relevance to cancer. Cancer Cell 8, 179–183. https://doi.org/10.1016/j.ccr.2005.08.008.

Hou, Y., Li, W., Sheng, Y., Li, L., Huang, Y., Zhang, Z., Zhu, T., Peace, D., Quigley, J.G., Wu, W., et al. (2015). The transcription factor Foxm1 is essential for the quiescence and maintenance of hematopoietic stem cells. Nat. Immunol. 16, 810–818. https://doi.org/10.1038/ni.3294.

Hu, M., Lu, Y., Zeng, H., Zhang, Z., Chen, S., Qi, Y., Xu, Y., Chen, F., Tang, Y., Chen, M., et al. (2021). MicroRNA-21 maintains hematopoietic stem cell homeostasis through sustaining the NF-kappaB signaling pathway in mice. Haematologica 106, 412–423. https://doi.org/10.3324/haematol.2019.236927.

Hu, M., Zeng, H., Chen, S., Xu, Y., Wang, S., Tang, Y., Wang, X., Du, C., Shen, M., Chen, F., et al. (2018). SRC-3 is involved in

(J) BM cells (1 × 10⁶) from WT or Srebf1c⁻/⁻ mice (CD45.2) coupled with BM cells (1 × 10⁶) from CD45.1⁺ mice were transplanted into lethally irradiated CD45.1⁺ recipients. One week after transplantation, recipients received vehicle or rapamycin (4 mg/kg) by i.p. injection every day for 12 weeks. The donor percentages in the recipients’ PB were analyzed by flow cytometry (n = 6–8).

(K) Schematic overview of the proposed model demonstrating the role of Srebf1c in regulating HSC homeostasis.

**p < 0.01.
maintaining hematopoietic stem cell quiescence by regulation of mitochondrial metabolism in mice. Blood 132, 911–923. https://doi.org/10.1182/blood-2018-02-831669.

Inoki, K., Li, Y., Zhu, T., Wu, J., and Guan, K.L. (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat. Cell Biol. 4, 648–657. https://doi.org/10.1038/ncb839.

Jacinto, E., Loeewith, R., Schmidt, A., Lin, S., Ruegg, M.A., Hall, A., and Hall, M.N. (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat. Cell Biol. 6, 1122–1128. https://doi.org/10.1038/ncb1183.

Kalaitzidis, D., Sykes, S.M., Wang, Z., Punt, N., Tang, Y., Ragu, C., Sinha, A.U., Lane, S.W., Souza, A.L., Clish, C.B., et al. (2012). mTOR complex 1 plays critical roles in hematopoiesis and Pten-loss-evoked leukemogenesis. Cell Stem Cell 11, 429–439. https://doi.org/10.1016/j.stem.2012.06.009.

Khoa, L.T.P., Tsan, Y.-C., Mao, F., Kremer, D.M., Sajjakulnukit, P., Zhang, L., Zhou, B., Tong, X., Bhanu, N.V., Choudhary, C., et al. (2020). Histone acetyltransferase MOF Blocks acquisition of quiescence in ground-state ESCs through activating fatty acid oxidation. Cell Stem Cell 27, 441–458.e10. https://doi.org/10.1016/j.stem.2020.06.005.

Lee, D.-E., Kuo, H.-P., Chen, C.-T., Hsu, J.-M., Chou, C.-K., Wei, Y., Sun, H.-L., Li, L.-Y., Ping, B., Huang, W.-C., et al. (2007). IKKβ suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. Cell 130, 440–455. https://doi.org/10.1016/j.cell.2007.05.058.

Lee, G., Jang, H., Kim, Y., Choe, S., Kong, J., Hwang, I., Park, J., Im, S., and Kim, J. (2019). SREBP1c-PAX4 Axis mediates pancreatic β-cell compensatory responses upon metabolic stress. Diabetes 68, 81–94. https://doi.org/10.23736/S0012-1878.18.30556-6.

Li, Z., Qian, P., Shao, W., Shi, H., He, X.C., Gogol, M., Yu, Z., Wang, Y., Qi, M., Zhu, Y., et al. (2018). Suppression of m(6)A reader Ythdf2 promotes hematopoietic stem cell expansion. Cell Res. 28, 904–917. https://doi.org/10.1038/s41422-018-0072-0.

Liang, G., Yang, J., Horton, J.D., Hammer, R.E., Goldstein, J.L., and Brown, M.S. (2002). Diminished hepatic response to fasting/re-feeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. J. Biol. Chem. 277, 9520–9528. https://doi.org/10.1074/jbc.M111421200.

Liang, R., Arif, T., Kalmykova, S., Kasianov, A., Lin, M., Menon, V., Qiu, J., Bernitz, J.M., Moore, K., Lin, F., et al. (2020). Restraining lysosomal activity preserves hematopoietic stem cell quiescence and potency. Cell Stem Cell 26, 359–376.e7. https://doi.org/10.1016. 2020.10.013.

Liu, L., Inoki, A., Fan, K., Mao, F., Shi, G., Jin, X., Zhao, M., Neya, G., Sun, S., Dou, Y., et al. (2019). ER associated degradation preserves hematopoietic stem cell quiescence and self-renewal by restricting mTOR activity. Blood 136, 2975–2986. https://doi.org/10.1182/blood.2019000063.

Ludikhuize, M.C., Meerlo, M., Gallego, M.P., Xanthakis, D., Burgaya Julia, M., Nguyen, N.T.B., Brombacher, E.C., Liv, N., Maurice, M.M., Paik, J.H., et al. (2020). Mitochondria define intestinal stem cell differentiation downstream of a FOXO/notch Axis. Cell Metab. 32, 889–900.e7. https://doi.org/10.1016/j.cmet.2020.10.005.

Mansell, E., Sigurdsson, V., Deltcheva, E., Brown, J., James, C., Miharada, K., Soneji, S., Larsson, J., and Enver, T. (2021). Mitochondrial potentiation ameliorates age-related heterogeneity in hematopoietic stem cell function. Cell Stem Cell 28, 241–256.e6. https://doi.org/10.1016/j.stem.2020.09.018.

Mendelson, A., and Frenette, P.S. (2014). Hematopoietic stem cell niche maintenance during homeostasis and regeneration. Nat. Med. 20, 833–846. https://doi.org/10.1038/nm.3647.

Morita, M., Gravel, S.P., Chenard, V., Sikstrom, K., Zheng, L., Alain, T., Gandin, V., Avizond, D., Arguello, M., Zakaria, C., et al. (2013). mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. Cell Metab. 18, 698–711. https://doi.org/10.1016/j.cmet.2013.10.001.

Nakagawa, M.M., and Rathinam, C.V. (2018). Constitutive activation of the canonical NF-kappaB pathway leads to bone marrow failure and induction of erythroid signature in hematopoietic stem cells. Cell Rep. 25, 2094–2109.e4. https://doi.org/10.1016.j.celrep.2018.10.071.

Normen, C., Figlia, G., Lebrun-Julien, F., Pereira, J.A., Trotzmueller, M., Kofeler, H.C., Rantanen, V., Wessig, C., van Deijk, A.L., Smit, A.B., et al. (2014). mTORC1 controls PNS myelination along the mTORC1-GLRXgamma-SREBP-lipid biosynthesis axis in Schwann cells. Cell Rep. 9, 646–660. https://doi.org/10.1016/j.celrep.2014.09.001.

Pinho, S., and Frenette, P.S. (2019). Haematopoietic stem cell activity and interactions with the niche. Nat. Rev. Mol. Cell Biol. 20, 303–320. https://doi.org/10.1038/s41580-019-0103-9.

Qian, P., He, X.C., Paulson, A., Li, Z., Tao, F., Perry, J.M., Guo, F., Zhao, M., Zhi, L., Venkatraman, A., et al. (2016). The dlk1-gtl2 locus preserves LT-HSC function by inhibiting the PI3K-mTOR pathway to restrict mitochondrial metabolism. Cell Stem Cell 18, 214–228. https://doi.org/10.1016/j.stem.2015.11.001.

Rao, T.N., Hansen, N., Hilfiker, J., Rai, S., Majewska, J.M., Lekovic, D., Gezer, D., Andina, N., Galli, S., Cassel, T., et al. (2019). JAK2 mutant hematopoietic cells display metabolic alterations that can be targeted to treat myeloproliferative neoplasms. Blood 134, 1832–1846. https://doi.org/10.1182/blood.2019000162.

Saxton, R.A., and Sahatini, D.M. (2017). mTOR signaling in growth, metabolism, and disease. Cell 168, 960–976. https://doi.org/10.1016/j.cell.2017.02.004.

Sinha, S., Dwivedi, T.R., Yengkhom, R., Bheemsetty, V.A., Abe, T., Kiyonari, H., VijayRaghavan, K., and Inamdar, M.S. (2019). Asrij/OClid1 suppresses CSNS-mediated p53 degradation and maintains mouse hematopoietic stem cell quiescence. Blood 133, 2385–2400. https://doi.org/10.1182/blood.2019000530.

Su, S., Tian, H., Jia, X., Zhu, X., Wu, J., Zhang, Y., Chen, Y., Li, Z., and Zhou, Y. (2020). Mechanistic insights into the effects of SREBP1c on hepatic stellate cell and liver fibrosis. J. Cell. Mol. Med. 24, 10063–10074. https://doi.org/10.1111/jcm.15614.

Suda, T., Takubo, K., and Semenza, G.L. (2011). Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell 9, 298–310. https://doi.org/10.1016/j.stem.2011.09.010.

Takubo, K., Goda, N., Yamada, W., Iritschishina, H., Ikeda, E., Kubota, Y., Shima, H., Johnson, R.S., Hirao, A., Suematsu, M., and Suda, T. (2010). Regulation of the HIF-1alpha level is essential for
hematopoietic stem cells. Cell Stem Cell 7, 391–402. https://doi.org/10.1016/j.stem.2010.06.020.

Takubo, K., Nagamatsu, G., Kobayashi, C.I., Nakamura-Ishizu, A., Kobayashi, H., Ikeda, E., Goda, N., Rahimi, Y., Johnson, R.S., Soga, T., et al. (2013). Regulation of glycolysis by pdk functions as a metabolic checkpoint for cell cycle quiescence in hematopoietic stem cells. Cell Stem Cell 12, 49–61. https://doi.org/10.1016/j.stem.2012.10.011.

Tohyama, S., Fujita, J., Hishiki, T., Matsuura, T., Hattori, F., Ohno, R., Kanazawa, H., Seki, T., Nakajima, K., Kishino, Y., et al. (2016). Glutamine oxidation is indispensable for survival of human pluripotent stem cells. Cell Metab. 23, 663–674. https://doi.org/10.1016/j.cmet.2016.03.001.

Umemoto, T., Hashimoto, M., Matsumura, T., Nakamura-Ishizu, A., and Suda, T. (2018). Ca(2+)-mitochondria axis drives cell division in hematopoietic stem cells. J. Exp. Med. 215, 2097–2113. https://doi.org/10.1084/jem.20180421.

Villegas, F., Lehalle, D., Mayer, D., Rittirsch, M., Stadler, M.B., Zinner, M., Olivieri, D., Vabres, P., Duplomb-Jego, L., De Bont, E.S.J.M., et al. (2019). Lysosomal signaling licenses embryonic stem cell differentiation via inactivation of Tfe3. Cell Stem Cell 24, 257–270.e8. https://doi.org/10.1016/j.stem.2018.11.021.

Wang, H., Diao, D.J., Shi, Z.C., Zhu, X.D., Gao, Y.W., Gao, S.R., Liu, X.Y., Wu, Y., Rudolph, K.L., Liu, G.H., et al. (2016). SIRT6 controls hematopoietic stem cell homeostasis through epigenetic regulation of Wnt signaling. Cell Stem Cell 18, 495–507. https://doi.org/10.1016/j.stem.2016.03.005.

Wang, N., Yin, J., You, N., Yang, S., Guo, D., Zhao, Y., Ru, Y., Liu, X., Cheng, H., Ren, Q., et al. (2021). TWIST1 preserves hematopoietic stem cell function via the CACNA1B/Ca2+/mitochondria axis. Blood 137, 2907–2919. https://doi.org/10.1182/blood.2020007489.

Wang, Y., Viscarra, J., Kim, S.-J., and Sul, H.S. (2015). Transcriptional regulation of hepatic lipogenesis. Nat. Rev. Mol. Cell Biol. 16, 678–689. https://doi.org/10.1038/nrm4074.

Xia, P., Wang, S., Ye, B., Du, Y., Li, C., Xiong, Z., Qu, Y., and Fan, Z. (2018). A circular RNA protects dormant hematopoietic stem cells from DNA sensor cGAS-mediated exhaustion. Immunity 48, 688–701.e7. https://doi.org/10.1016/j.immuni.2018.03.016.

Yamashita, M., and Passegue, E. (2019). TNF-alpha coordinates hematopoietic stem cell survival and myeloid regeneration. Cell Stem Cell 25, 357–372.e7. https://doi.org/10.1016/j.stem.2019.05.019.

Yecies, J.L., Zhang, H.H., Menon, S., Liu, S., Yecies, D., Lipovsky, A.I., Gorgun, C., Kwiatkowski, D.J., Hotamisligil, G.S., Lee, C.H., and Manning, B.D. (2011). Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. Cell Metab. 14, 21–32. https://doi.org/10.1016/j.cmet.2011.06.002.
Supplemental Information

*Srebf1c* preserves hematopoietic stem cell function and survival as a switch of mitochondrial metabolism

Yukai Lu, Zihao Zhang, Song Wang, Yan Qi, Fang Chen, Yang Xu, Mingqiang Shen, Mo Chen, Naicheng Chen, Lijing Yang, Shilei Chen, Fengchao Wang, Yongping Su, Mengjia Hu, and Junping Wang
Supplemental Information:

Supplemental Figures and Legends

Figure S1, related to Figure 1

(A-C) The flow cytometric gating strategies of (A) Lineage\(^{-}\) (Lin\(^{-}\)) cells, Lin\(^{+}\) cells, myeloid progenitors (MPS; Lin\(^{-}\) Sca1\(^{-}\) c-Kit\(^{+}\)), LSKs (Lin\(^{-}\) Sca1\(^{+}\) c-Kit\(^{+}\)), long-term HSCs (LT-HSCs; Lin\(^{-}\) Sca1\(^{+}\) c-Kit\(^{+}\) CD34\(^{-}\) Flk2\(^{-}\)), short-term HSCs (ST-HSCs; Lin\(^{-}\) Sca1\(^{+}\) c-Kit\(^{+}\) CD34\(^{+}\) Flk2\(^{-}\)), multipotent progenitors (MPPs; Lin\(^{-}\) Sca1\(^{-}\) c-Kit\(^{+}\) CD34\(^{-}\) Flk2\(^{-}\)), SLAM-HSCs (Lin\(^{-}\) Sca1\(^{+}\) c-Kit\(^{+}\) CD150\(^{-}\) CD48\(^{-}\)), (B) granulocyte monocyte progenitors (GMPs; Lin\(^{-}\) CD127\(^{-}\) Sca1\(^{-}\) c-Kit\(^{+}\) CD16/32\(^{-}\) CD34\(^{-}\)), megakaryocyte erythroid progenitors (MEPs; Lin\(^{-}\) CD127\(^{-}\) Sca1\(^{-}\) c-Kit\(^{+}\) CD16/32\(^{-}\) CD34\(^{-}\)), common myeloid progenitors (CMPs; Lin\(^{-}\) CD127\(^{-}\) Sca1\(^{-}\) c-Kit\(^{+}\) CD16/32\(^{-}\) CD34\(^{-}\)), and common lymphoid progenitors (CLPs; Lin\(^{-}\) CD127\(^{-}\) Sca1\(^{med}\) c-Kit\(^{med}\)); (C) T cells (CD3\(^{e}\)), B cells (B220\(^{+}\)) and myeloid cells (Gr-1\(^{+}\) Mac-1\(^{+}\)). (D) The counts of white blood cell (WBC), red blood cell (RBC), platelet (PLT), lymphocyte (LYMPH), monocyte (MONO) in the peripheral blood (PB) of WT and Srebf1c\(^{-}\) mice (n = 10).
Figure S2. Srebfc deletion leads to increased extramedullary hematopoiesis. Related to Figure 2.

Flow cytometric analysis of the percentage of LSKs in the PB of WT and Srebfc−/− mice (n = 5). **P < 0.01.
Figure S3. Srebf1c deficiency decreases the quiescence and increases the apoptosis of HSCs. Related to Figure 3

(A) Flow cytometric analysis of cell-cycle distribution of ST-HSCs, MPPs, MPs from WT and Srebf1c−/− mice (n = 5). (B) Flow cytometric analysis of BrdU incorporation in ST-HSCs, MPPs, MPs from WT and Srebf1c−/− mice (n = 5). (C) Flow cytometric analysis of apoptosis in ST-HSCs, MPPs, MPs from WT and Srebf1c−/− mice (n = 5). **P < 0.01.
Figure S4, related to Figure 4

(A) Schematic of homing assay (left) and the homing efficiency (right) of LSKs from WT or Srebff1c−/− mice (n = 5). (B) In vitro colonies formation analysis of 180 single-cell LT-HSC sorted from WT and Srebff1c−/− mice (n = 3) (C-D) Flow cytometric analysis of the frequency of LT-HSCs in G0, G1 and S/G2/M phases from (C) CD45.1+ recipients transplanted with WT or Srebff1c−/− (CD45.2) BM cells and (D) WT or Srebff1c−/− recipients (CD45.2) transplanted with CD45.1+ BM cells (n = 5-8) at 16 weeks after transplantation. *P < 0.05, **P < 0.01.
Figure S5. Sreb1c knockout results in increased proliferation-related signatures and mitochondrial activity in HSCs. Related to Figure 5.

(A) GSEA of proliferation-related signatures (G2M checkpoint, DNA replication and cell cycle) in RNA-seq data from WT and Sreb1c<sup>-/-</sup> LSKs. (B) qPCR analysis of the mRNA expression of Fasn and Acc1 in WT and Sreb1c<sup>-/-</sup> LT-HSCs (n = 3) (C) Representative flow cytometric plots of cell size (left) and the percentage of large cells (right) in LT-HSCs, ST-HSCs and MPPs from WT and Sreb1c<sup>-/-</sup> mice (n = 10). (D) Flow cytometric analysis of mitochondrial mass in SLAM-HSCs from WT and Sreb1c<sup>-/-</sup> mice by MTG staining (n = 5). (E) Flow cytometric analysis of mitochondrial membrane potential in SLAM-HSCs from WT and Sreb1c<sup>-/-</sup> mice by TMRM staining (n = 5). (F) Relative mtDNA copy number (mtDNA/nDNA) in LT-HSCs and Lin<sup>+</sup> cells sorted from WT and Sreb1c<sup>-/-</sup> mice (n = 3). (G) qPCR analysis of the mRNA expression of mitochondria-related genes (Cyc1, Atp4a, Cox5a, Cox6a2, Ndufc1 and Idh3a) in WT and Sreb1c<sup>-/-</sup> LT-HSCs (n = 3). (H) Flow cytometric analysis of glucose uptake in MPs, LSKs and LT-HSCs from WT and Sreb1c<sup>-/-</sup> mice by 2-NBDG staining (n = 5). (I, J) Relative pyruvate...
dehydrogenase (PDH) activity (I) and lactate dehydrogenase (LDH) activity (J) in LT-HSCs and Lin+ cells sorted from WT and Srebf1c−/− mice (n = 3). (K) Relative pyruvate levels in LT-HSCs and Lin+ cells sorted from WT and Srebf1c−/− mice (n = 5). *P < 0.05, **P < 0.01.
Figure S6. Srebf1c-null HSCs display an increased activity of mTOR signaling, with no alteration in p-Akt and TSC2 level. Related to Figure 6.

(A, B) Flow cytometric analysis of the expression of (A) p-S6 (Ser235, Ser236) and (B) p-Akt (S473) in MPs, LSKs and LT-HSCs from WT and Srebf1c−/− mice (n = 5). (C) Flow cytometric analysis of the expression of TSC1 in SLAM-HSCs from WT and Srebf1c−/− mice (n = 5). (D) qPCR analysis of the mRNA expression of Tsc2 in WT and Srebf1c−/− LT-HSCs (n = 3). (E) Flow cytometric analysis of the expression of TSC2 in MPs, LSKs and LT-HSCs from WT and Srebf1c−/− mice (n = 5). ** P < 0.01.
Figure S7. Inhibition of mTOR activation or scavenging ROS improves HSC defects in Srebf1c-deleted mice. Related to Figure 7.

(A, B) WT and Srebf1c<sup>−/−</sup> mice were administered i.p. vehicle or rapamycin (4 mg/kg) every other day for 2 weeks. The levels of (A) glucose uptake and (B) BrdU incorporation in LT-HSCs of WT and Srebf1c<sup>−/−</sup> mice were analyzed by flow cytometry (n = 5). (C) BM cells (1×10<sup>6</sup>) from WT or Srebf1c<sup>−/−</sup> mice (CD45.2) coupled with BM cells (1×10<sup>6</sup>) from CD45.1<sup>+</sup> mice were transplanted into lethally irradiated CD45.1<sup>+</sup> recipients. One week after transplantation, recipients were received vehicle or rapamycin (4 mg/kg) by intraperitoneal injection every day for 12 weeks. The donor percentages in LT-HSCs of recipients were analyzed by flow cytometry (n = 6-8). (D-I) WT or Srebf1c<sup>−/−</sup> mice were injected i.p. with NAC (100 mg/kg) daily for 14 days, then BM cells were isolated for flow cytometric analysis or competitive transplantation with an equal number of CD45.1<sup>+</sup> BM cells. After transplantation, recipients were fed with vehicle or NAC (1 mg/ml) in drinking water for 16 weeks. (D) Schematic of NAC treatment and competitive BMT. (E-H) Flow cytometric analysis of the (E) ROS levels, (F) absolute number (two femurs and
tibias), (G) apoptosis and (H) cell-cycle distribution of LT-HSCs from WT and Srebf1c−/− mice treated with vehicle or NAC for 14 days (n = 5). (I) The percentage of WT or Srebf1c−/− (CD45.2) donor-derived cells in the PB of recipients (CD45.1) after vehicle or NAC treatment (n = 6-7). **P < 0.01.
Supplemental Tables

Table S1. Antibodies used in flow cytometry, western blotting and immunofluorescence

Table S2. Primers for mRNA expression, mtDNA copy number and ChIP analysis
Supplemental Experimental Procedures

5-FU, rapamycin or NAC treatment
WT and Srebf1c−/− mice were treated i.p. with 5-FU (150 mg/kg; Sigma) every week for three times, and then the survival rate was recorded. For inhibiting mTOR pathway, mice were injected i.p. with rapamycin (4 mg/kg; MedChemExpress, Monmouth Junction, NJ, USA) every other day. For scavenging ROS, mice were administrated with NAC (Sigma) through intraperitoneal injection (100 mg/kg) or drinking water (1 mg/ml).

Single LT-HSC culture
Single LT-HSC was directly sorted into U-bottom 96-well plate containing 100 μl Methocult™ GF M3434 medium (Stemcell Technologies, Vancouver, Canada). After 12 days of incubation at 37°C, size of colonies was evaluated using an inverted microscope.

qPCR
To measure the expression levels of genes, cells were sorted and then total RNA was extracted using a RNAqueous Kit (Ambion) according to the manufacturer’s instructions. Next, RNA (50 ng) was reversely transcribed into cDNA using a PrimeScript™ RT reagent Kit (TaKaRa, Shiga, Japan). Finally, qPCR reactions were conducted using a SYBR® Premix EX Taq™ II Kit (TaKaRa). Data were normalized relative to Gapdh. To quantify relative DNA copy number of mitochondria, genomic and mitochondrial DNA from sorted cells were isolated using a DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), followed by qPCR detection. The relative expression of NADH dehydrogenase subunit 1 (mtDNA; mt-Nd1) was normalized to β2-microglobulin (nDNA; B2m). The primer sequences are listed in Table S2.

Transmission electron microscopy (TEM)
LSKs sorted from WT and Srebf1c−/− mice were centrifuged and fixed in 2.5% glutaraldehyde overnight at 4°C. Subsequently, samples were sequentially post-fixed, dehydrated and embedded. Ultrathin sections were cut and stained with uranyl acetate and lead citrate. Finally, the sections were imaged using a JEM-1400 TEM (JEOL, Tokyo, Japan).

PDH activity, LDH activity, pyruvate and lactate levels analysis
LT-HSCs or Lin+ cells sorted from WT and Srebf1c−/− mice were lysed, and then intracellular PDH activity, LDH activity, pyruvate and lactate concentration were measured using the Pyruvate Dehydrogenase Activity Colorimetric Assay Kit (BioVision, Milpitas, CA, USA), the LDH Cytotoxicity Colorimetric Assay Kit (BioVision), the Pyruvate Assay Kit (BioVision), or the Lactate Assay Kit (BioVision) according to the manufacturer’s instructions.

Western blotting
LSKs (5×10⁵, pooled from 5-6 mice) freshly isolated by flow cytometry were lysed in RIPA buffer (Beyotime) on ice for 30 minutes. After quantification and denaturation, proteins were separated on a 10% polyacrylamide gel and transferred to a polyvinylidene difluoride membranes (Millipore, Massachusetts, USA). Blots were blocked with QuickBlock™ Blocking Buffer (Beyotime) at room temperature for one hour, and then were incubated with anti-TSC1 (Novus
Biologicals, Littleton, Colorado, USA) or anti-GAPDH (Abcam). After incubated with rabbit anti-sheep IgG-HRP (Abcam) or goat anti-rabbit IgG-HRP (Beyotime), detection was conducted by using an ECL solution (US Everbright® Inc, Suzhou, China). The relative expression of protein was quantified by analyzing the grey intensity in Fiji software (National Institutes of Health, Bethesda, MD, USA). Further details of Western blotting antibodies are provided in Table S1.

**Immunofluorescence**

LT-HSCs sorted from WT and Srebflc−/− mice were cytopun on Poly-L-lysine coating slides, and then fixed for 15 minutes with 4% paraformaldehyde. After washed three times with PBS, cells were permeabilized by 0.5% Triton X-100 for 15 minutes. Next, samples were incubated with anti-TSC1 (Novus Biologicals) and anti-TOMM2O (Abcam) overnight at 4°C, washed with PBS and stained with donkey anti-rabbit secondary antibody (Abcam) or donkey anti-sheep secondary antibody (Invitrogen, Carlsbad, CA, USA). Finally, images were captured using a Zeiss LSM780 NLO (Carl Zeiss, Jena, Germany) confocal microscope. Further details of immunofluorescence antibodies are provided in Table S1.

**ChIP assays**

ChIP assays were performed using the Cleavage Under Target & Tagmentation method. In brief, LSKs (1×10^5) sorted from WT and Srebflc−/− mice were incubated with concanavalin A-coated magnetic beads (ConA beads) at room temperature. After incubation with anti-SREBP1 (Santa Cruz Biotechnology, Dallas, USA) or mouse IgG (Abcam) overnight at 4°C, the cells were treated with goat anti-mouse IgG (Abcam) at room temperature for 1 hour. Then, the hyperactive Tn5 transposon fused with Protein A/Protein G was precisely targeted to cut the DNA sequence near the target protein through the mediation of the respective incubation of the primary antibody, the corresponding secondary antibody and Protein A/Protein G. For DNA fragmentation, the DNA was incubated with Tagmentation buffer at 37°C for 1 hour. After DNA extraction, amplification and purification, the library was directly used for qPCR. The primer sequences are listed in Table S2.