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LKB1 regulates quiescence and metabolic homeostasis of hematopoietic stem cells

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Abstract

The capacity to fine-tune cellular bioenergetics with the demands of stem cell maintenance and regeneration is central to normal development and aging and to organismal survival during periods of acute stress. How energy metabolism and stem cell homeostatic processes are coordinated is not well understood. LKB1 acts as an evolutionarily conserved regulator of cellular energy metabolism in eukaryotic cells and functions as the major upstream kinase to phosphorylate AMPK and 12 other AMPK-related kinases 1–3. Whether LKB1 regulates stem cell maintenance remains unknown. Here we show that LKB1 plays an essential role in hematopoietic stem cell (HSC) homeostasis. We demonstrate that ablation of *Lkb1* in adult mice results in severe pancytopenia and subsequent lethality. Loss of *Lkb1* leads to impaired survival and escape from quiescence of HSCs, resulting in exhaustion of the HSC pool and marked reduction of HSC repopulating potential *in vivo. Lkb1* deletion impacted cell proliferation in HSCs, but not more committed compartments, pointing to context specific functions for LKB1 in hematopoiesis. The adverse impact of *Lkb1* deletion on hematopoiesis was predominantly cell-autonomous and mTORC1-independent and involves multiple mechanisms converging on mitochondrial apoptosis and possibly down-regulation of PGC-1 coactivators and their transcriptional network which plays critical roles in mitochondrial biogenesis and function. Thus, LKB1 serves as an essential regulator of HSCs and hematopoiesis, and more generally, points to the critical importance of coupling energy metabolism and stem cell homeostasis.

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Author Contributions

B. G. and R. A. D. designed the experiments, interpreted the data, and wrote the manuscript. B. G., S. J., J. H., L. Z., E. F. performed experiments. Y. L. and L. C. conducted the microarray and promoter analyses. E. S. and S. C. contributed reagents. L. C. and Y. A. W. contributed to the writing of the manuscript.

Author Information

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HSCs function to replenish the blood system and maintain hematopoietic homeostasis in response to either physiological or imposed stress demands 4–6. To explore the role of LKB1 in various aspects of HSC biology, we assessed the impact of somatic deletion of *Lkb1* in the mouse adult hematopoietic system using *Rosa26-CreERT2* deletor mice 7. In this model, the treatment of adult mice with tamoxifen results in complete deletion of *Lkb1* in hematopoietic organs (Supplementary Fig. 1a), and associated reductions of phosphorylation of AMPK Thr172 and AMPK substrate Acetyl-CoA carboxylase (ACC) Ser79 (Supplementary Fig. 1b). Strikingly, within 30 days post completing tamoxifen injection (DPI), all tamoxifen-treated *Lkb1 L/L, Rosa26-CreERT2* mice (hereafter designated *Lkb1* KO) exhibited constitutional signs of weight loss (Supplementary Fig. 2a), lethargy, hunched posture, and ultimately death (Fig. 1a); in contrast, tamoxifen-treated *Lkb1 +/+, Rosa26-CreERT2 or Lkb1 L/L* mice (collectively hereafter designated *Lkb1* WT) remained viable and healthy (Fig. 1a).

Somatic deletion of *Lkb1* led to pancytopenia within 1 week after tamoxifen treatment (7 DPI) – evidenced by reduced weight of spleen and thymus, and reduction of the absolute cell number of bone marrow, spleen and thymus (Supplementary Figs. 2b–f). *Lkb1* KO mice also developed acute anemia as evidenced by marked decline in red blood cell, hemoglobin and hematocrit counts (Supplementary Figs. 2g–i). While anemic, *Lkb1* KO mice had increased non-fasting blood glucose levels (Supplementary Fig. 3), making unlikely that the *Lkb1* KO anemia phenotype derives from a profound systemic deficiencies in glucose availability. Further analysis revealed severe reductions in *Lkb1* KO Ter119^{+} cells and erythroid progenitors at all developmental stages (Fig. 1b, Supplementary Figs. 4a–b). Consistent with the pancytopenia phenotype described above, we observed a decline of all hematopoietic lineages examined in the *Lkb1* KO mice, including platelets (Supplementary Fig. 4c), Gr-1^{+}/Mac-1^{+} cells (Fig. 1c and Supplementary Fig. 4d), B cells (Fig. 1d and Supplementary Fig. 4e), and T cells (Supplementary Fig. 4f). To study the underlying mechanism of the multi-lineage defects associated with *Lkb1* deletion, we examined the cell survival status of various lineages in *Lkb1* WT and KO mice given the important role of LKB1 in the maintenance of cell survival under energy stress in other cellular contexts 8,9. Indeed we observed an increase of cleaved Caspase-3 in *Lkb1* KO bone marrow, spleen and thymus samples (Supplementary Fig. 5). Further Annexin V/7AAD analysis revealed increased apoptosis in myeloid, erythroid and B cell populations in *Lkb1* KO bone marrow cells (Fig. 1e). In summary, *Lkb1* deficiency leads to severe pancytopenia phenotype and impairs the cell survival of multiple lineages.

Next we examined HSC and hematopoietic progenitor populations in *Lkb1* KO mice. Compared with WT controls, serial analysis of *Lkb1* KO HSC-enriched LSK cells (Lin^{-}, Sca-1^{+}, c-Kit^{+}) and long-term HSCs (LT-HSCs; CD34^{-} Flt-3^{-} LSKs) in bone marrow showed an acute increase at 1 DPI, but a subsequent decrease in numbers from 4 DPI and thereafter (Figs. 2a–b). BrdU labeling experiment revealed significantly increased percentage of BrdU positive cells in *Lkb1* KO LSK cells (Fig. 2c and Supplementary Figs. 6a–b). Notably, *Lkb1* deletion increased cell proliferation only in LSK and LSK CD34^{-} cells, but not whole bone marrow and mature lineage cells (Fig. 2b), suggesting an HSC-specific role for LKB1 in the regulation of cell quiescence. The more pronounced function

of LKB1 in HSC compartment also aligns well with its more prominent expression levels in HSCs relative to other more committed compartments (Supplementary Figs. 6c–d). Furthermore, 7-AAD/Annexin-V staining of LSK population showed increased apoptosis in *Lkb1* KO LSK cells (Supplementary Figs. 6e–f). Together, our results suggest that LKB1 functions to maintain HSC quiescence and survival and that *Lkb1* inactivation leads to transient expansion, yet subsequent decline, of bone marrow HSCs.

We next performed competitive and noncompetitive transplantation assays to examine the impact of *Lkb1* deficiency on HSC repopulating capability *in vivo* (Supplementary Figs. 7a–b). In the competitive transplantation experiments, we observed that *Lkb1* KO transplants showed markedly diminished repopulating capability relative to WT controls (Fig. 2d, and Supplementary Figs. 8a–c). In the noncompetitive experiments, all recipient mice reconstituted with *Lkb1L/L, Rosa26-CreERT2* bone marrow cells died within 60 DPI (Supplementary Fig. 8d) with anemia, pancytopenia defects (data not shown), and increased HSC cell cycle entry and subsequent decline (Supplementary Figs. 8e–f). These data collectively indicate that LKB1 exerts a predominant cell-autonomous impact on hematopoietic repopulating potential and homeostasis.

To understand the mechanisms underlying LKB1-directed regulation of HSC homeostasis, we assessed the impact of pharmacological inhibition of mTORC1 signaling on the *Lkb1* KO phenotype, given that mTORC1 serves as a key downstream surrogate of LKB1-AMPK signaling 3 and also plays critical roles in the regulation of HSC homeostasis 10–14. Daily rapamycin or vehicle protocols were initiated to *Lkb1* or *Tsc1* WT and KO mice at the time of tamoxifen treatment 12. Rapamycin treatment significantly rescued multi-lineage defects, HSC cycling increase and expansion phenotypes in *Tsc1* KO mice, but had minimal effect on these phenotypes in *Lkb1* KO mice (Figs. 3a–c) despite confirmation of abolished S6 phosphorylation in sorted bone marrow $B220^+$ cells, $Mac1^+$ cells and $CD34^-$ LSK cells from *Lkb1* KO mice (Supplementary Fig. 9). Thus, unlike TSC1, LKB1 regulates hematopoiesis via an mTORC1-independent pathway. Finally, administration of metformin, a known AMPK activator, did not rescue bone marrow/thymus cellularity decline, LSK transient expansion/subsequent depletion phenotype and lineage defects in *Lkb1* KO mice (Supplementary Fig. 10). These data, together with AMPK activator A-769662 treatment data from Gurumurthy et al and AMPK KO mice analyses from Nakada et al, suggest that either multiple AMPK-related kinases (including AMPK) cooperatively mediate LKB1 function in HSC homeostasis or non-AMPK dependent processes are operative.

The above observations prompted transcriptome analysis of sorted LSK cells from *Lkb1* WT and KO bone marrows at 1 DPI to gain further mechanistic insight of LKB1 regulation of HSC homeostasis (Supplementary Fig. 11a). Ingenuity pathway analysis of 570 significantly differentially expressed genes revealed significant enrichment of genes involved in G1/S cell cycle checkpoint regulation (Supplementary Fig. 11b, and Supplementary Table 1) including up-regulation of Cyclin D1, Cyclin D2, Cyclin E1, Cdc25A, E2F1, Cdk4 and Skp2, which would serve to synergistically promote cell cycling of *Lkb1* KO LSKs. Most notably, there was prominent representation of LXR/RXR, VDR/RXR and PPAR metabolism pathways (Supplementary Fig. 11b). To identify key networks regulated by LKB1 in a TSC-mTORC1-independent manner, we further conducted comparative analysis of *Lkb1* and

Tsc1 12 HSC transcriptome datasets (Supplementary Fig. 11a), which revealed that, although G1/S cell cycle checkpoint was enriched in both datasets, the LXR/RXR, VDR/RXR and PPAR metabolism pathways were distinctively enriched in the *Lkb1* transcriptome dataset (Supplementary Fig. 11c). In addition, promoter analysis of *Lkb1* HSC transcriptome dataset identified E2F, nuclear respiratory factor 1 (NRF1) and PPARγ motifs as the most significantly enriched promoter binding elements in *Lkb1* LSK transcriptome dataset (1.4x, 2.1x and 1.4x, respectively). The link to PPARγ and NRF1 is notable given that peroxisome proliferator-activated receptor-coactivators, PGC-1α and PGC-1β, are the principal transcriptional coactivator for PPARγ and NRF1 15, and that the PGC-1s are regulated by LKB1 16.

Prompted by this observation, we next investigated whether *Lkb1* deletion impacted on PGC-1, a master transcriptional regulator of mitochondrial biogenesis 17, and its associated biological processes in the hematopoietic system. We found the expression levels of both PGC-1α and PGC-1 β were down-regulated in *Lkb1* KO LSKs (Fig. 4a), which coincided with decreased mitochondrial membrane potential and DNA content in *Lkb1* KO LSKs at 4 DPI (Figs. 4b–c). Interestingly, we observed increased mitochondrial DNA content in *Lkb1* KO LSKs at later time point (10 DPI), possibly reflecting compensatory (secondary) effects that occur in the wake of mitochondriopathy (Fig. 4c). Finally, we found that the basal ATP levels in spleen and thymus were profoundly decreased in *Lkb1* KO mice (Fig. 4d). Although our data raises the possibility that *Lkb1* deficiency and associated dysregulation of PGC-1 impair mitochondrial function, the presence of apoptosis in these *Lkb1*$^{-/-}$ HSCs does not allow us to exclude the possibility that mitochondrial dysfunction reflects in part an ongoing apoptotic process caused by *Lkb1* deletion.

In conclusion, our results reveal an essential role of LKB1 in the maintenance of HSC homeostasis. Somatic deletion of *Lkb1* in the hematopoietic system impairs HSC quiescence and survival and leads to metabolic catastrophe, resulting in pancytopenia and rapid animal death. Mechanistically, we propose that LKB1 maintains HSC homeostasis through multiple mechanisms governing mitochondrial function, cell survival, and cell cycle regulation via LKB1 regulation of AMPK and other AMPK-related kinases and their downstream effectors (including PGC-1). Our findings align with those of Nakada et al and Gurumurthy et al. and provide a broad framework to understand the integration of energy signaling and mitochondrial physiology in the maintenance of HSC homeostasis.

Methods Summary

The *Lkb1 L/L* mice were described previously 18. *Rosa26CreERT2* mice were provided by A. Berns 7. *Lkb1 L/+*, *Rosa26CreERT2*$^{+}$ mice were backcrossed 6 generations into C57BL/Ka-CD45.2:Thy-1.1 background, which were then intercrossed to generate mice of the desired genotypes. Recipients in transplantation assays were adult C57BL/Ka-CD45.1:Thy-1.2 mice (Jackson Laboratory). Tamoxifen treatment, phenotypic analyses of mice, rapamycin administration, flow cytometric analysis, and transplantation assays were performed as previously described 12. All animal manipulations were performed with Harvard/DFCI's Institutional Animal Care and Use Committee (IACUC) approval. For microarray analysis, the RNA from sorted LSK cells (10,000–20,000) was extracted and the

cDNA was analyzed on the Affymetrix 430 2.0 platform. Quantitative real-time PCR was performed on the Stratagene Mx3000P utilizing the Quantitative SYBR Green PCR kit. Full methods and the associated references are available in the online version of the paper.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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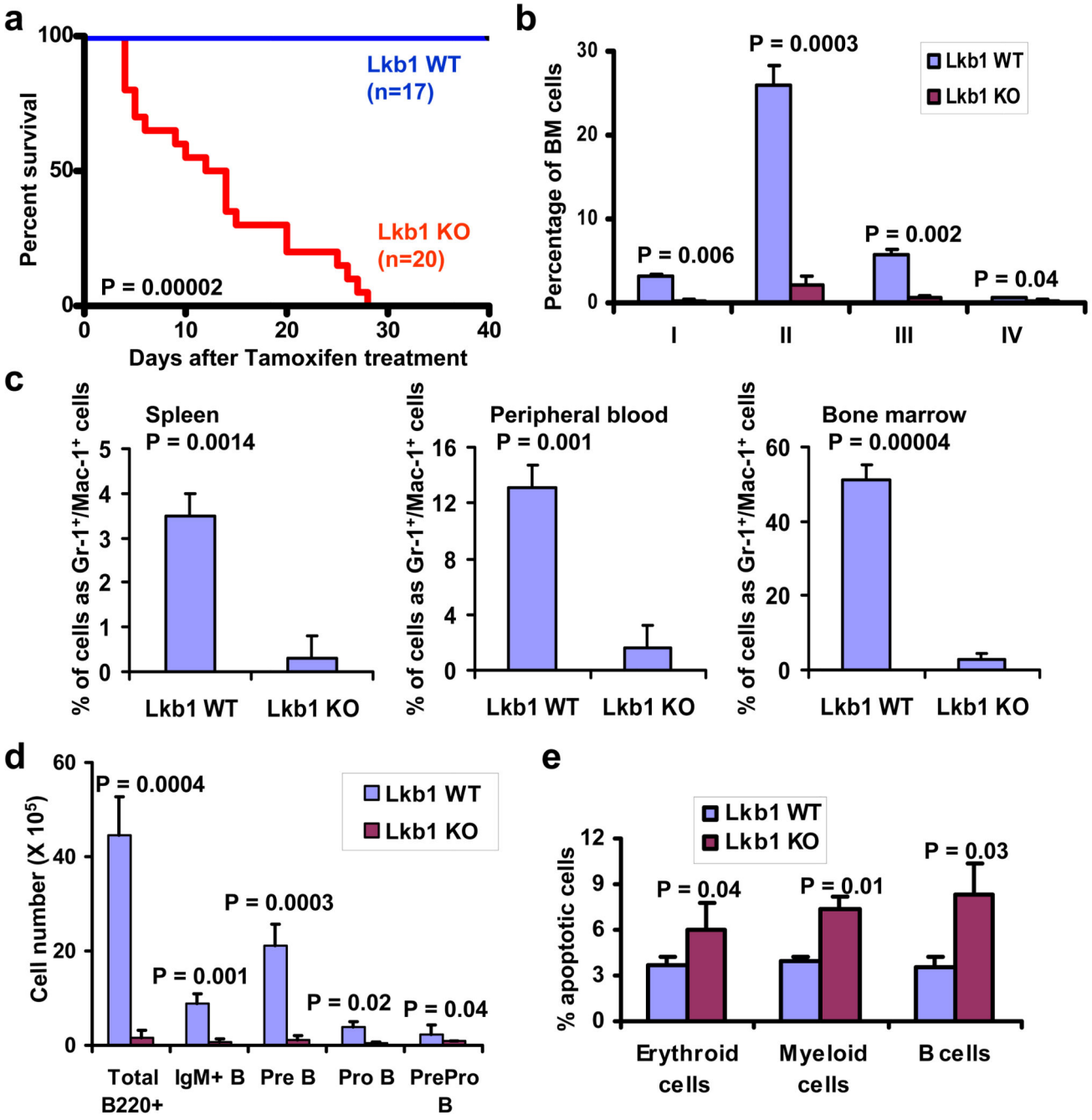

Figure 1. *Lkb1* deletion leads to severe pancytopenia phenotype

a, Kaplan-Meier survival analysis of *Lkb1* WT and KO mice. **b, c,** The percentages of erythroid lineage from bone marrow (**b**) and Mac-1$^+$/Gr-1$^+$ cells (**c**) in *Lkb1* WT and KO mice at 5 DPI. **d,** The numbers of bone marrow B cell lineage at 7 DPI from *Lkb1* WT and KO mice. **e,** The percentages of apoptotic cells in erythroid, myeloid and B cells of *Lkb1* WT and KO bone marrow cells at 3 DPI. n > 3 (**b**–**e**).

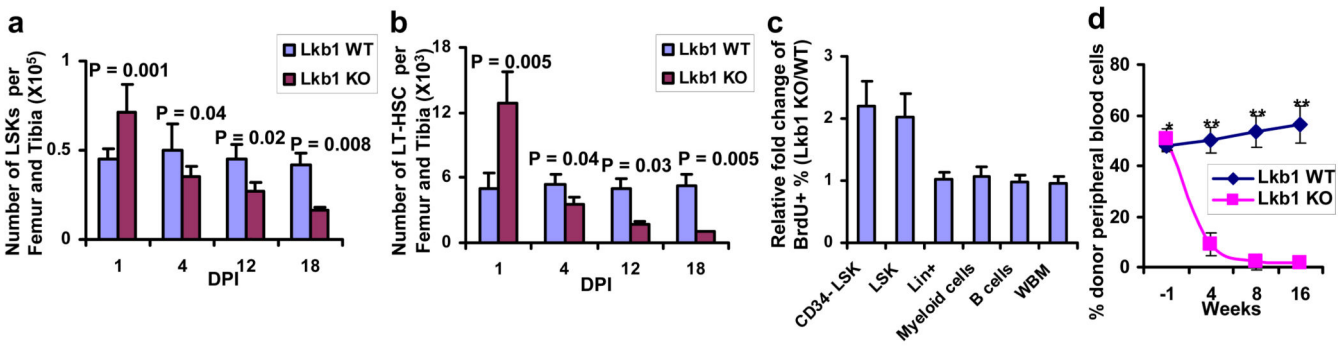

Figure 2. *Lkb1* ablation results in reduced HSC reserves and decreased repopulating potential

a, b, The numbers of LSK cells (**a**) and LT-HSCs (**b**) at various DPIs in *Lkb1* WT and KO bone marrows. **c,** The relative fold changes (*Lkb1* KO/WT) of the percentages of $BrdU^+$ cells from various hematopoietic cell lineages in *Lkb1* WT and KO bone marrows at 1 DPI. n = or > 3 at each time point (**a–c**). **d,** The percentages of donor-derived cells in peripheral blood by CD45 staining in 1:1 competitive transplantation. n = 15. *: P > 0.4; **: P < 0.01.

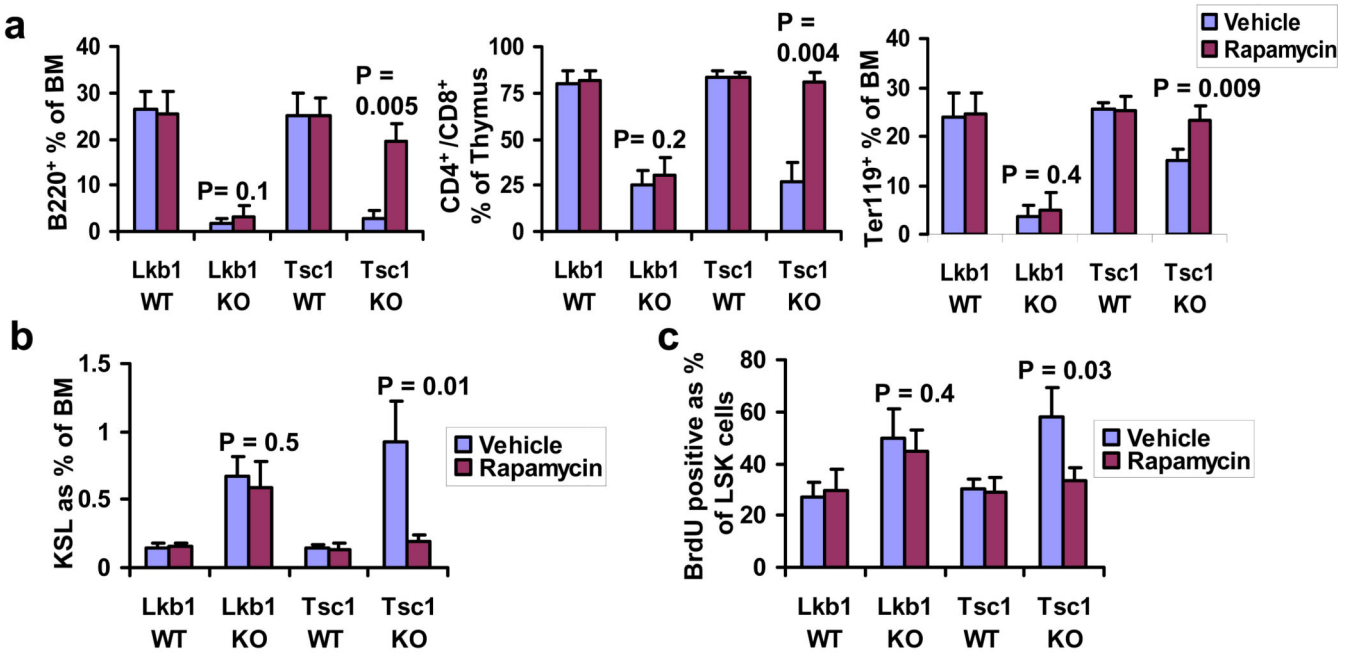

Figure 3. LKB1 regulation of hematopoiesis is TSC-mTORC1-independent

a, b, c, The percentages of $B220^+$ and $Ter119^+$ populations in bone marrow and thymic $CD4^+/CD8^+$ cells (**a**), bone marrow LSK cells (**b**), and $BrdU^+$ bone marrow LSK cells (**c**) from the mice indicated. n = 3 (**a–c**).

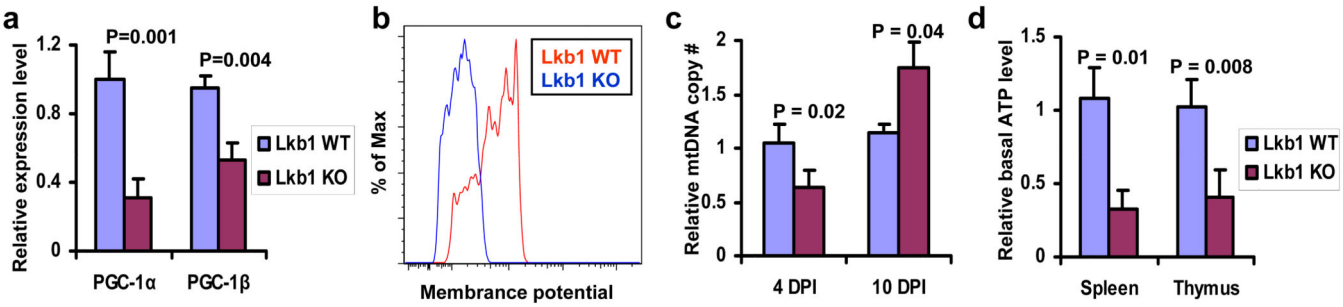

Figure 4. *Lkb1* deletion diminishes mitochondrial biogenesis and energy production in the hematopoietic system

a, b, c, The relative expression levels of PGC-1α/β at 2 DPI (**a**), the mitochondrial membrane potential at 4 DPI (**b**), and the relative mitochondria DNA copy numbers at 4 and 10 DPI (**c**) of *Lkb1* WT and KO bone marrow LSKs. **d,** The relative basal ATP levels in spleen and thymus from *Lkb1* WT and KO mice at 4 DPI. n = 3 (**a–d**).