Free fatty-acid transport via CD36 drives β-oxidation-mediated hematopoietic stem cell response to infection

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Acute infection is known to induce rapid expansion of hematopoietic stem cells (HSCs), but the mechanisms supporting this expansion remain incomplete. Using mouse models, we show that inducible CD36 is required for free fatty acid uptake by HSCs during acute infection, allowing the metabolic transition from glycolysis towards β-oxidation. Mechanistically, high CD36 levels promote FFA uptake, which enables CPT1A to transport fatty acyl chains from the cytosol into the mitochondria. Without CD36-mediated FFA uptake, the HSCs are unable to enter the cell cycle, subsequently enhancing mortality in response to bacterial infection. These findings enhance our understanding of HSC metabolism in the bone marrow microenvironment, which supports the expansion of HSCs during pathogenic challenge.
The maintenance of hematopoiesis is reliant on the lifelong self-renewal and differentiation of the hematopoietic stem cells (HSCs) into all lineages of mature blood cells. Hematopoiesis is a dynamic balance between the opposing cell fates of self-renewal and initiation of hematopoietic differentiation. The HSCs, while predominantly quiescent, rapidly enter the cell cycle in response to infection, this rapid expansion of white blood cells in response to pathogenic stress underpins the mammalian response to infection. At present, the mechanisms by which HSC metabolism is regulated in response to the challenges of pathogenic stimuli are not fully understood.

The bone marrow (BM) microenvironment regulates the production of both hematopoietic and non-hematopoietic cells for the maintenance of blood production under normal and stressed conditions. HSC metabolism is finely balanced between glycolysis and oxidative phosphorylation (OXPHOS), to maintain the intrinsic needs of the cell within the constraints imposed by the microenvironment. Under “steady-state” conditions HSCs reside in a hypoxic niche where quiescent HSC have low mitochondrial activity and favor anaerobic glycolysis to generate the energy requirements for cell maintenance. After chemotoxic or pathogenic stimulation HSCs move out-of-quiescence and rapidly switch their metabolic profile towards an increase in mitochondrial activity and OXPHOS-dependent ATP generation. This switch allows differentiating cells to meet their altered and higher metabolic energy demands associated with expansion and differentiation.

Fatty-acid oxidation is utilized as an energy source by both primitive HSCs and more-committed progenitors to aid self-renewal and differentiation. Moreover, inhibiting fatty-acid oxidation has been shown to reduce stem cell capability. In a malignant setting, the leukemic stem cell (LSC) has been shown to interact with its microenvironment resulting in lipolysis of marrow adipose tissue (MAT), fueling the LSC via fatty-acid oxidation. Specifically, the LSCs expressing the fatty-acid transporter CD36 demonstrated high levels of fatty-acid oxidation, providing those LSC with a survival advantage. In addition, we have also shown that free fatty acids are acquired by acute myeloid leukemia (AML) blasts to enhance their proliferation in vitro and in vivo through a mechanism that increased β-oxidation. This leads us to hypothesize that the β-oxidation-dependent metabolic switch in leukemia has its “origins” in the physiology of the HSC response to infection.

The majority of invasive non-typhoidal salmonella infections are due to Salmonella Typhimurium (S. typhimurium), and have become a prominent cause of bloodstream infection in African adults and children, associated with 20–25% fatality. We have recently reported that in the context of the challenge of acute S. typhimurium infection, the BM microenvironment drives rapid HSC and leukocyte expansion necessary for host survival, through a process dependent on mitochondria transfer into the HSC from tissue-resident BM stromal cells.

Therefore, through studies using S. typhimurium and its outer membrane lipopolysaccharide (LPS) to model acute bacterial infection, we aim to understand if and how the mammalian response to infection involves the acquisition of free fatty acids by HSC in the BM microenvironment. Furthermore, we look to elucidate the mechanisms by which FA transport occurs and how it facilitates the immunometabolic changes required for rapid leukocyte expansion in response to bacterial infection.

**Results**

Infection with S. typhimurium drives long-chain fatty-acid uptake in HSC. It has previously been shown that serum FFA levels are increased in response to infection. Here, we observe increased levels of FFA in the serum of mice treated with S. typhimurium (72 hours) and LPS (16 hours) based on serum IL-6 levels. Moreover, blocking IL-6 inhibits LPS-induced serum FFA levels in vivo (Supplementary 1a–e). In addition, we show that significantly increased cell cycling of HSC in response to LPS and S. typhimurium is occurring at 16 hours and 72 hours post exposure, respectively (Supplementary Fig. 1f, g). To investigate real-time fatty-acid uptake by hematopoietic cells in response to infection we developed an in vivo transplant model in which we transduced CD45.1 lineage negative, CD117-positive cells with firefly luciferase (LK+ff). Adoptive transfer of LK+ff cells into CD45.2 animals was performed (Fig. 1a). Post transplantation animals were injected with the β-luciferin molecule conjugated to a long-chain free fatty acid (LFA-luc) by a cleavable disulfide bond and imaged using bioluminescence. This probe is stable outside of the cell but is reduced by glutathione following lipid uptake into the cell. If there is LFA uptake the luciferin reacts with the luciferase which can be seen by bioluminescent imaging. One-week later, the mice were treated with LPS and 16 hours later we injected the mice with the LFA-luc probe. Live animal imaging confims activation of luciferase in the BM compartment following LPS treatment, demonstrating in vivo, that long-chain FFA is taken up by hematopoietic cells in response to LPS (Fig. 1c, d). However, it is noted that no background bioluminescence is present in the control group, which we believe is due to the signal threshold not being reached.

Next, we looked to determine which hematopoietic stem and/or progenitor cells (HSPC) have increased lipids during infection. Mice were treated with S. typhimurium for 72 hours then sacrificed (Fig. 1e). Analysis of LSK, MPP, HSC, ST-HSC, and LT-HSC populations (Fig. 1f) showed an increase in intracellular neutral lipid staining at 72 hours compared to control non-infected animals (Fig. 1g). LPS treatment for 16 hours also increased intracellular neutral lipid staining in the HSPC populations (Fig. 1h and supplementary Fig. 1h). To investigate if this was uptake of FFA, LK cells were isolated from the BM of mice infected with 72 hours S. typhimurium or treated with LPS for 16 hours and incubated with a BODIPY-dodecanoic acid fluorescent fatty-acid for 30 minutes (long-chain FFA linked to bodipy). LK cells from S. typhimurium or LPS-treated mice had an increased uptake of FFA when compared to LK cells from untreated animals (Fig. 1i). Fluorescent microscopy images confirm an increase in FFA in the LSK cells from S. typhimurium (72 hours) infected mice (Fig. 1j). Moreover, following 16 hours LPS treatment the LSK, HSC, ST-HSC, and LT-HSC populations had increased uptake of long and short-chain FAs (Supplementary Fig. 2a–c). Together, these experiments show that HSC, MPP, and LSK cells all acquire FFA in the context of bacterial infection.

Infection increases OXPHOS and dependency on β-oxidation in HSPC. To understand the metabolic changes occurring in the HSC in response to infection we studied LSK cells isolated from animals infected with S. typhimurium for 72 hours, or treated with LPS for 16 hours. Seahorse metabolic flux analysis measuring oxygen consumption rates (OCR) confirmed increased OXPHOS levels in LSK cells from LPS (16 hours) treated and S. typhimurium (72 hours) infected C57BL/6J mice (Fig. 2a, b). Extracellular acidification rate (ECAR) was also measured and showed no change in glycolysis following S. typhimurium infection or LPS treatment (Fig. 2c and Supplementary 3a). Cells can utilize glucose, glutamine, and/or fatty acids to generate ATP and
metabolites to support increased cellular activities. We used the Seahorse XF Mito Fuel Flex Test to monitor the dependency on fatty acids as a source of energy in LSK cells from LPS (16 hours) treated animals. Results confirm an increased dependency on fatty-acid oxidation (FAO) compared to control LSK cells (Fig. 2d). To determine the role of β-oxidation in OXPHOS we treated animals with LPS then isolated LSK cells after 16 hours and treated the cells ex vivo with etomoxir (Eto) a β-oxidation inhibitor specifically an inhibitor of mitochondrial CPT1, an enzyme located on the outer mitochondrial membrane responsible for the catalyzing the first step of FAO. Seahorse XF Mito stress test showed that Eto inhibited the LPS-induced increase in
CD36 regulates long-chain free fatty-acid uptake in HSC in response to infection. Several membrane proteins have been identified to facilitate the trafficking of lipids into and out of cells, including CD206, CD36, fatty-acid-binding proteins (FABPs), and fatty-acid transport proteins (FATPs; also known as solute carrier family 27 (SLC27)). The FATP family includes six members including CD206, CD36, fatty-acid-binding proteins (FABPs), and fatty-acid transport proteins (FATPs). Several membrane proteins have been identified to facilitate the trafficking of lipids into and out of cells, including CD206, CD36, fatty-acid-binding proteins (FABPs), and fatty-acid transport proteins (FATPs; also known as solute carrier family 27 (SLC27)). The FATP family includes six members including CD206, CD36, fatty-acid-binding proteins (FABPs), and fatty-acid transport proteins (FATPs). To test the functional importance of CD36 in the uptake of FFA and its impact on HSC expansion in response to infection, animals were pre-treated with the CD36 inhibitor SSO before injection with LPS (Fig. 4c). LKs isolated from animals treated with SSO and LPS had reduced uptake of FFA compared with LPS alone (Fig. 4d). Moreover, HSPCs isolated from these animals pre-treated with SSO before stimulation with LPS had a reduced lipid content, lower basal and maximal respiration, and reduced cycling when compared to animals treated with LPS alone (Supplementary Fig. 4d–f and Fig. 4e).

To confirm the effects of pharmacological inhibition of CD36 in response to infection was consistent with the genetic knockout of CD36 we treated WT (CD36+/+) and CD36 knockout (CD36−/−) animals with LPS for 16 hours (Fig. 4f). Unlike the WT CD36+/+ animals, we found LPS-treated CD36−/− mice had no increase in FFA uptake, lipid content, or HSC cycling compared to control CD36−/− mice (Fig. 4g, h and Supplementary Fig. 4g, h). In a similar way, 72 hours after inoculation with S. typhimurium, fluorescent microscopy demonstrated no increase in lipid content in the LSK cells from infected CD36−/− mice compared to control CD36−/− mice. (Fig. 4i and Supplementary Fig. 5a).

To understand the impact of CD36 expression on HSC metabolism in response to infection we infected WT CD36+/+ and CD36−/− animals with S. typhimurium 72 hours. LKs were then isolated and analyzed using the Seahorse XF Mito stress test. In contrast to LKs cells from WT CD36+/+ mice, we found CD36−/− LKs had an increased basal ECAR but no changes in basal OCR in response to S. typhimurium infection (Fig. 4j, k and Supplementary Fig. 5b). Maximal OCR respiration was also inhibited in the infected CD36−/− LKs cells compared with infected WT CD36−/− LKs cells (Supplementary Fig. 5b). Moreover, CD36−/− LKs cells from control and LPS-treated mice have reduced dependency on β-oxidation (Supplementary Fig. 5c).

To investigate the role of CD36 on fatty-acid uptake by hematopoietic cells in response to infection we transduced CD36−/− (CD45.2) lineage negative, CD117-positive (LK) cells were isolated and transduced with firefly luciferase (LK+FF) and transplanted into WT CD45.1 animals. B LK cells were Imaging using bioluminescence to confirm engrafment. We mice were with control PBS for 16 hours then treated with FFA-SS-luc and imaging using bioluminescence Representative images of control and LPS-treated mice. D Densitometry of the bioluminescent images in (C) to determine fluorescence intensity in the vehicle and LPS-treated animals. n = 4. Schematic diagram of experiment in which C57BL/6/J mice were infected with S. typhimurium (Sal) for 72 hours and analyzed for LSK, MPP, HSC, ST-HSC, and LT-HSC populations by flow cytometry. F The gating strategy used to identify the LSK, MPP, HSC ST-HSC, LT-HSC populations are shown. G Lipid content (Bodipy 493/503 mean fluorescence intensity (MFI)) was assessed by flow cytometry from control and S. typhimurium (Sal) (72 hours) treated mice. n = 7 in each group. H C57BL/6/J mice were treated with 1 mg/kg LPS for 16 hours, the bone marrow was extracted, and the cells were analyzed by flow cytometry for lipid content (Bodipy 493/503 MFI) n = 6 in each group. I C57BL/6 J mice were infected with S. typhimurium (Sal) for 72 hours or LPS for 16 hours. Long-chain fatty-acid (LCFA) uptake was measured using the QBT assay. n = 5 in each group. J C57BL/6/J mice were infected with S. typhimurium (Sal) for 72 hours. Representative live-cell fluorescent microscopy images of LSK cells isolated from the mice, Scal membrane stain (red), Bodipy 493/503 (green), and Hoechst 33342 (blue). Quantification of Bodipy 493/503 fluorescence in LSK cells from images shown, 20 LSK cells from five mice in each condition. Data shown are means ± SD. The Mann-Whitney U test (two-tailed) was used to compare between treatment groups *p < 0.05 “p < 0.01 ***p < 0.001. Source data are provided as a Source Data file.
in response to LPS (Supplementary Fig. 5e and Fig. 4m). Together, these data show that CD36 on HSCs is essential for the uptake of FFA in response to infection.

**FFA uptake through CD36 is an essential component of HSC expansion in response to infection.** To determine if the uptake of FFA response is required for HSC expansion we isolated LK cells from WT CD36−/− (CD45.1) mice and transplanted them into CD36−/− (CD45.2) animals (Fig. 5a). Therefore, these animals were CD36−/− but had a WT CD36+/+ hematopoietic system. Engraftment of CD36+/+ (CD45.1) cells are shown in Supplementary Figs. 6a–e and 7a. We found that CD36 expression was elevated in the HSC from the CD36−/− mice treated with LPS (Fig. 5b). Moreover, following LPS treatment we observed increased uptake of FFA, increase in lipid content, and increased expansion of HSCs, similar to the WT animal response (Fig. 5c, d and Supplementary Fig. 7b–d). In addition, LK cells from transplanted WT CD36−/− (CD45.1) into CD36−/− (CD45.2) animals reversed the metabolic phenotype observed in CD36−/− mice in response to LPS with an increase in OXPHOS, both basal and maximal mitochondrial respiration (Fig. 5e, f).
Finally, we wanted to understand the importance of CD36 in the hematopoietic compartment in response to *S. typhimurium* infection. WT (CD45.1) mice received an adoptive transfer of either WT CD36$^{+/+}$ (CD45.2) or CD36$^{-/-}$ (CD45.2) LK cells. These transplanted mice were termed WT(CD36$^{-/-}$/-) for CD36 knockout into WT and WT(CD36$^{+/+}$/+) into WT. Post engraftment the mice were infected with *S. typhimurium* for 4 days (Fig. 5g and Supplementary Fig. 7e). *S. typhimurium* infected WT(CD36$^{-/-}$/-) transplanted animals showed enhanced mortality and weight loss compared with infected WT(CD36$^{+/+}$/+) transplanted animals (Fig. 5h, i). Further analysis evidenced increased liver injury in WT(CD36$^{-/-}$/-) compared with WT(CD36$^{+/+}$/+) after *S. typhimurium* infection, as shown by the increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum samples and the wide areas of necrosis observed in Hematoxylin & Eosin (H&E)-stained histological liver sections (Fig. 5j, k). We then validated the differentiation ability of CD36$^{-/-}$/- HSCs at steady-state and during infection. CD36$^{-/-}$/- mice were treated with control PBS (CD36$^{-/-}$/-con) or LPS (CD36$^{-/-}$/-LPS) for 16 hours, the mice were sacrificed, the HSCs were isolated and transplanted into WT (CD45.1) mice (Supplementary Fig. 8a). We found no differences in the myeloid/lymphoid ratio in the peripheral blood or the
**Fig. 3** Infection increases dependency on β-oxidation. a Schematic diagram of experimental design in which C57BL/6 J mice were infected with *S. typhimurium* (Sal) for 72 hours and 10 mg/kg/day Eto (Eto) or 1 mg/kg LPS for 16 hours and 10 mg/kg Eto. The bone marrow was extracted, and the cells were analyzed by flow cytometry for LSK and HSC. b Percentage of cycling HSC and LSK as measured by Ki67-positive cells after 16 hours of S. typhimurium and 10 mg/kg/day Eto treatment. n = 5 mice in each group. c Percentage of cycling HSC and LSK as measured by Ki67-positive cells after 16 hours of 1 mg/kg LPS and 10 mg/kg Eto treatment. n = 4 mice in each group. d Number of LSKs and HSCs leg after 72 hours S. typhimurium and Eto treatment. n = 5 in each group. e Number of LSKs and HSCs per leg after 16 hours of LPS and Eto treatment. n = 5 mice in each group. f C57BL/6 J mice were treated with 1 mg/kg LPS for 16 hours or S. typhimurium for 72 h, HSC were FACS-sorted from control and LPS-treated animals. RNA was analyzed for CPTIA gene expression by qPCR. n = 4 in each group. g Schematic diagram of experimental design. WT CD45.1 lineage negative, CD117-positive (LK) cells were transduced with a CPTIA knockdown lentivirus (LK(CPTIA KD)) were transplanted into WT CD45.2 animals. Post engraftment mice were treated with 1 mg/kg LPS for 16 hours. h The bone marrow was extracted, and analyzed by flow cytometry for the LSK and HSC population. i Percentage of cycling HSC and LSK as measured by Ki67-positive cells after 16 hours of 1 mg/kg LPS treatment. n = 5 mice in each group. j Number of LSKs and HSCs per leg after 16 hours of LPS treatment. n = 5 mice in each group. Data shown are means ± SD. The Mann-Whitney U test (two-tailed) was used to compare between two treatment groups and the Kruskal-Wallis test was followed by Dunn’s multiple comparison post hoc test to compare between three treatment groups. *p < 0.05 **p < 0.1. Source data are provided as a Source Data file.

**Discussion**

Here we report that HSCs actively uptake long-chain FFA in response to acute bacterial infection. This process facilitates an increased reliance, within the HSCs from glycolytic metabolism towards β-oxidation and a proliferation phenotype. FFA uptake occurs after the onset of the transcriptional changes to the fatty-acid transporter CD36. Furthermore, we identified that without surface CD36, HSC is not able to switch from glycolytic metabolism towards β-oxidation and does not enter the cell cycle, leading to a higher susceptibility and increased mortality to infection. Overall, these results provide insights into the metabolic changes in the hematopoietic system which underpin leukocyte expansion and the mammalian response to infection.

The transition from steady-state to emergency hematopoiesis is established to involve a complex remodeling and interplay between hematopoietic and non-hematopoietic cells of the BM microenvironment mediated by cytokines and growth factors. Therefore, the defects caused by CD36- in HSC may only be evident during stress. Together these data show that CD36 is an important mediator for the HSC response to infection.

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**Frequency of different progenitor (CMP GMP or MEP) cells in the BM of CD36−/−con or CD36−/−LPS transplant mice, 12 weeks post engraftment (Supplementary Figs. 8b, c and 9a–d). Therefore, the defects caused by CD36- in HSC may only be evident during stress. Together these data show that CD36 is an important mediator for the HSC response to infection.**

**Rates of fatty-acid oxidation leads to stem cell differentiation through a mechanism mediated by peroxisome proliferator-activated receptor δ.** Our study finds that during infection HSCs take up FFA as a result of upregulation of the fatty-acid translocase CD36 on the cell surface. CD36 belongs to a family of proteins that bind, transport, and take up long-chain fatty acids or function as regulators of these processes. In the past several years, the membrane protein CD36 has been extensively studied for its role in facilitating fatty-acid uptake and oxidation and implicated in the pathophysiology of the heart and liver and associated with dysfunctional fatty-acid metabolism. In fact, it is because CD36 is expressed on many different cell types, and also because of the plurality of the disease phenotypes related in some way to CD36 function, that in order to determine the cell-autonomous effects of CD36 function in HSC we decided to develop the model system using knockout CD36 in HSCs and then transplanting these CD36−/− HSC cells back into a wild-type animal, rather than use CD36 knockout animals for our studies. In contrast, our present study identifies CD36 upregulation in HSC which mediates fatty-acid uptake and allows HSC expansion facilitating the response to infection. It is also important to understand the role of CD36 non-autonomous mechanism. How CD36 is regulated in HSC is not fully known, but others have shown that C/EBPα and C/EBPβ can directly up-regulate CD36 gene transcription through a C/EBP-responing element at the proximal promoter. Moreover, both C/EBPα and C/EBPβ have been shown to mediate steady-state and emergency granulopoiesis respectively. At the epigenetic level, the enhancers and promoters of CD36 are subject to non-coding RNA. Differentiation of HSC [CD36 negative] into CD36 expressing erythroid precursors is associated with global chromatin modification patterns. Specifically, Cui and colleagues linked the H3K4me3 mark at the CD36 promoter to transcriptional activity. More recently, these studies in the context of the current work suggest that CD36 forms part of a tightly regulated metabolic switch central to the mammalian haematopoietic response to infection.

This study does not ask the question about the source of FFA. However, BM adipose tissue (MAT), is biologically active energy storage and endocrine organ and accounts for ~70% of BM volume in adult humans. Moreover, BM adipocytes are known to increase with age in humans and rodents. These adipocytes are not merely passive occupants of the BM but are now appreciated to be actively involved in processes linked to bone metabolism, osteoporosis, inflammation, and regulation of the hematopoietic niche. In addition, BM adipocytes have been shown to support the proliferation of tumors located in the BM including AML, multiple myeloma, and metastatic solid
However, what stimulus is responsible for lipolysis to occur in response to infection is still unknown. There are some data to suggest that endogenous IL-6 is important in regulating both uptake and release of fatty acids from adipocytes. Here we show an association between induced IL-6 and FFA uptake in HSC. Moreover, we observed that blocking IL-6 with a monoclonal antibody reduced the serum content of FFA in response to LPS, suggesting that IL-6 is involved in regulating lipolysis in response to infection.

In conclusion, we report that adaptations in FFA uptake and FAO functionally support the hematopoietic response to bacterial infection. Furthermore, in doing so we provide a base for further studies investigating how benign and malignant stem cells in other tissues utilize FFA in response to cellular stress, and specifically whether the provision of FFA by adipocytes in the physiologic response to infection, is altered in individuals with particular vulnerabilities with respect to infection, including older people and those with obesity.

**Methods**

**Animals.** C57BL/6J mice (CD45.2), were purchased (Charles River Massachusetts, United States). B6.129S1-Cd36tm1Mfe/J (CD36−/−) were purchased from The Jackson Laboratory (Bar Harbour, ME, USA). Mice were individually ventilated and housed under specific pathogen-free conditions in a 12/12-hour light/dark cycle with food and water.
provided ad libitum. The room temperature for mice was 22 °C and the relative humidity is kept at between 45% and 65%. All animal work used in this study was carried out in accordance with regulations set by the UK Home Office and the Animal Scientific Procedures Act 1986. Mice were used at 8–12 weeks of age and both genders were used for experiments with the exception of mice that were used for transplantation which 3–4-week-old mice were used.

**Cell isolation and preparation.** BM isolation was prepared by isolating the tibia, femur, and pelvis of each mouse. The bone was cut in the middle and placed in a 0.5 ml Eppendorf tube in which a hole was made to allow the removal of the BM, placed in an intact 1.5 ml Eppendorf and centrifuged 1000 × g for 6 seconds to collect the BM cells. The BM pellet from each mouse was pooled and washed in PBS, with the red cells being lysed using 1× red blood cell lysis buffer (ThermoFisher, Waltham, MA, USA) and centrifuged at 400 × g for 5 min, and the pellet was resuspended in antibody cocktails in PBS.

**BM transplantation.** For the FFA-luciferase allograft mouse model C57BL/6J and CD36−/− mice expressing the CD45.2 allele antigen were used in the transplant experiments. C57BL/6J and CD36−/− mice were killed, BM was isolated, lineage depleted followed by CD117 enrichment using the LD Cell Select enrichment kit (LK cells). LK WT CD36−/− mice were then injected into the tail vein of 3–4-week-old PepCboy mice by intravenous injection which had been preconditioned with busulfan for 25 mg/kg/day for 3 days prior to transplantation. Post engraftment the animals were treated with 1 mg/kg LPS or control PBS for 16 hours and sacrificed. The BM was extracted and analyzed by flow cytometry for cell cycling and metabolic changes. Basal OCR normalized to rotenone. n = 5. *Schematic diagram of experimental design. **Mice were injected LPS for 16 hours then treated with FFA-luciferin-luc and imaged using bioluminescence (FFA-luciferin-LPS).** Densitometry of the bioluminescent images to determine fluorescence intensity. n = 4 for each group. The Mann–Whitney U test (two-tailed) was used and the Kruskal–Wallis test followed by Dunn’s multiple comparison post hoc test was used *p < 0.05 **p < 0.01. Source data are provided as a Source Data file.

**Fig. 4 CD36 regulates long-chain free fatty-acid uptake in HSC in response to infection.** a Heat map of fatty-acid transporter genes differentially expressed by HSCs from control, S. typhimurium (Sal), and LPS-treated animals. b Flow cytometry analysis of CD36 expression in the HSC of S. typhimurium and LPS-treated animals. n = 5 in each group. c C57BL/6J mice were pre-treated with SSO for one hour before treatment with LPS for 16 hours. n = 5 in each group. d The cells from control, LPS, or SSO and SSO-treated animals were isolated and long-chain fatty-acid (LCFA) uptake was measured using the QBT assay. n = 5 in each group. e Percentage of cycling HSCs as measured by Ki67-positive cells after pre-treatment followed by LPS treatment. n = 5. f Schematic diagram of the experiment. g The LK cells from LPS-treated CD36+/− or WT (CD36+/+) mice were isolated and long-chain fatty-acid (LCFA) uptake was measured using the QBT assay. n = 5. h Percentage of cycling HSCs from LPS-treated CD36+/− or WT (CD36+/+) mice as measured by Ki67-positive. n = 5 mice in each condition. i CD36+/− and CD36−/− mice were treated with S. typhimurium the animals were killed and the LSK population was isolated by FACS, OCR was measured. n = 5. j Basal ECAR compared to basal OCR levels before and after treatment with LPS or S. typhimurium. Basal OCR normalized to rotenone. n = 5. *Schematic diagram of experimental design. **Mice were injected LPS for 16 hours then treated with FFA-S5-luc and imaged using bioluminescence (FFA-luciferin-LPS).** Densitometry of the bioluminescent images to determine fluorescence intensity. n = 4 for each group. The Mann–Whitney U test (two-tailed) was used and the Kruskal–Wallis test followed by Dunn’s multiple comparison post hoc test was used *p < 0.05 **p < 0.01. Source data are provided as a Source Data file.

**FFA-luciferin assay.** Once engrafted the FFA-luciferase allograft mouse model was intraperitoneally injected with 100 μl of 200 μM FFA-S5-luc (SwissLumix Sarl, Switzerland) (0.014 mg/mouse) bound to 0.1% (w/v) bovine serum albumin (BSA) in PBS (a pentadecanoic acid, 16 carbon long-chain fatty-acid 15-carboxyl pentadecylic acid) immediately prior to imaging. Luminescence images were acquired with a 2-minute exposure (Bruker). The following week these animals were then treated with 1 mg/kg LPS for 16 hours. The mice were then intraperitoneally injected with 100 μl of 200 μM FFA-S5-luc (0.014 mg/mouse) bound to 0.1% (w/v) BSA in PBS immediately prior to imaging. Luminescence images were acquired with a 2-minute exposure (Bruker). For all transplantation experiments, engraftment was checked by CD45.1/2 expression on differentiated cells by blood sampling 8–12 weeks post transplantation. Four weeks post engraftment animals were treated.

**Analysis of CPT1A in vivo.** CPT1A is an enzyme located on the outer mitochondrial membrane and responsible for catalyzing the first step during mitochondrial fatty-acid oxidation. C57BL/6J mice were left untreated or infected with 100 μl of 1 × 10^8 CFU S. typhimurium (SL1344- JH3009) by oral gavage or infected with S. typhimurium by oral gavage and treated with etoxomix interperitoneally, etoxomix was repeatedly administered every 24 hrs. After 72 hours of S. typhi-murium infection mice were killed by exposure to CO₂. The BM was extracted for flow cytometry-based lineage analysis. C57BL/6J mice were also pre-treated with 50 mg/kg etoxomix interperitoneally for 1 hour followed by 1 mg/kg LPS interperitoneally after 16 hours the mice were killed, and the BM was extracted for flow cytometry-based lineage analysis of the LSK and HSC populations.

**FFA in serum.** C57BL/6J mice were left untreated or infected with 100 μl of 1 × 10^8 CFU S. typhimurium (SL1344- JH3009) by oral gavage for 72 hours. C57BL/6J mice were treated with LPS or control PBS for 16 hours. IL-6 inhibitor (clone MOPS-20F3) was used in combination with LPS. The mice were anesthetized and 600 μl blood was taken by cardiac puncture. The blood was spun at 1600 × g for 10 mins to remove cells, the supernatant was then spun again at 16,000 × g for 5 mins to collect serum. The serum was analyzed for free fatty acids using Plasma Fatty Acid and Glycerol Detection Kits (Zenzio, US) as per the manufacturer’s instructions.
**Fatty-acid uptake assay.** C57Bl/6 J or CD36+/+ or CD36−/− or transplant mice were interperitoneally injected with 1 mg/kg LPS after 16 hours the mice were sacrificed. The BM extracted, and the mouse Lineage negative cells were enriched using a direct cell lineage depletion kit and CD117+ enrichment kit (Miltenyi Biotec, Germany). In all, 5 × 10^4 LK cells were incubated with a long-chain fatty-acid 4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Dodecanoic Acid (BODIPY™ FL C12) (1 μM, Invitrogen) at room temperature for 20 min, washed twice in 1× PBS and centrifuged at 400 × g for 5 minutes before resuspending in PBS in a glass-bottom 96-well plate and read on a plate reader at 558/568 on a BMG Labtech microplate reader.

**Flow cytometry and cell sorting.** Antibody cocktails were prepared in MACs buffer and incubated with BM cells for at least 30 min at 4 °C. Experiments using 4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY 493/503), the cells were incubated with BODIPY 493/503 (1 μM, Invitrogen) at room temperature for 20 min, washed twice in 1× PBS and centrifuged at 400 × g for 5 minutes before staining with antibody cocktail. For experiments using Ki67 the BM cells were incubated with an antibody cocktail prior to fixing and permeabilization using the FIX & PERM™ Cell Permeabilization Kit (ThermoFisher, Waltherm, MA, USA) as per the manufacturer’s instructions. The cells were then stained with Anti-Ki-67-FITC, human and mouse (Miltenyi Biotec, Germany) for 20 min centrifuged at 400 × g for 5 mins before resuspending in PBS. For flow cytometric cell sorting of BM cell populations pellet was resuspended in antibody mix and cells were sorted directly into lysis buffer. Flow cytometry was carried out using FACSCanto II flow cytometer (BD Bioscience) and cell sorting was performed on a BD FACSMelody (BD Bioscience). Data were collected using BD FACS Diva software 8.0.1. Data were analyzed using FlowJo 10.7.0 (TreeStar, Ashland, OR, USA). See figures for specific gating strategies.

**Flow cytometry antibodies were purchased from Biolegend, Miltenyi Biotec and ThermoFisher. Lineage cocktail PacBlue anti-mouse Biologend Catalog number:133310 Lot number: B289722 (dilution 1:50). Components include anti-mouse CD3, clone 17A2 (dilution 1:100); anti-mouse Ly-6G/Ly-6C, clone RB6-8C5, anti-mouse CD45, clone 30-F11 (dilution 1:100); and anti-mouse CD117, clone 7B1 (dilution 1:100).**
Fig. 5 FFA uptake through CD36 is an essential component of HSC expansion in response to infection. a Schematic diagram of experimental design. CD36+/−/ CD45.1 lineage negative, CD117-positive cells were isolated and transplanted into CD36−/−/ CD45.2 animals. Post engraftment mice were treated with 1 mg/kg LPS for 16 hours and the bone marrow cells were analyzed by flow cytometry. b Flow cytometry analysis of CD36 expression (CD36 mean fluorescence intensity (MFI)) in the HSC from the transplant mice following 1 mg/kg LPS (16 hours) treatment. n = 5 mice in each group. c The LK cells were isolated and long-chain fatty-acid (LCFA) uptake was measured using the QBT assay. n = 5 mice in each group. d Percentage of cycling HSCs as measured by Ki67-positive cells from transplant mice after 16 hours of LPS. Basal OCR normalized to rotenone. n = 5 mice in each group. e Basal (normalized to rotenone) and maximal mitochondrial respiration in LK cells from control vs. LPS 16 hours treated transplanted mice. n = 5 mice in each group. g CD36+/−, CD45.2 or CD36−/−/ CD45.2 lineage negative, CD117-positive cells were isolated and transplanted into WT CD45.1 mice these were termed WT+/−/CD36− or WT−/−/CD36. Post engraftment mice were treated with S. typhimurium for 96 h. h Kaplan–Meier survival curve. n = 5 mice in each group. I Weight loss was analyzed. n = 5 mice in each group. j Levels of circulating alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum following 96 hours of S. typhimurium treatment. n > 5 in each group. k Livers were isolated and sectioned and stained with hematoxylin and eosin. (Magnification: H, ×63). n = 5 mice in each group. Data shown are means ± SD. The Mann–Whitney U test (two-tailed) was used to compare between treatment groups. *p < 0.05; **p < 0.01. Source data are provided as a Source Data file.

S. typhimurium and LPS-elicited stressed hematopoiesis. Glycerol stock of S. typhimurium (SL1344-JH3009) was a kind gift from Dr. Isabelle Hautefort (Quadram Institute Biosciences, Norwich). The stock was plated on Luria Broth agar plates and the colonies were inoculated and grown overnight into 5 ml of Luria Broth with 0.3 M NaCl (LBS). The overnight culture was then diluted 1:100 in LBS and grown until the culture optical density (AOD600) of 1.2–1.4 (late exponential phase). This is the time point where SPI1 invasion genes are turned on in S. typhimurium. The bacterial culture was then centrifuged at 3000 × g for 7 minutes before washing bacterial cells twice in 25 ml of sterile DPBS at room temperature. Finally, resuspend the bacterial cells in sterile DPBS at a concentration of 1 × 10^6 CFU per 100 μl of DPBS (known that DOD600 1.26 corresponds to 7.53 × 10^5 CFU/ml). C57Bl/6 or CD36−/− or CD36+/− mice were treated with streptomycin (20 mg/ml) 24 hr prior to S. typhimurium infection. Mice were then left untreated or infected with 100 μl of 1 × 10^6 CFU S. typhimurium (SL1344-JH3009) by oral gavage for 72 hours. C57Bl/6, CD36−/−, or transplanted mice were treated with seahorse metabolic analysis or transplanted mice were treated with 10 mg/kg Etomoxir intraperitoneally for 2 hours. The C57Bl/6 mice were also subjected to pre-treatment of 40 mg/kg SSO intraperitoneally or 10 mg/kg Etomoxir intraperitoneally for 1 hour followed by 1 mg/kg LPS. The mice were sacrificed by exposure to CO2, and the BM was analyzed by flow cytometry, cell sorting, and seahorse metabolic analysis.

Liver histology. Liver tissues were harvested and immediately fixed in 10% neutral formalin and embedded in paraffin blocks 24 hours later. Tissue blocks were sectioned, dehydrated, and hydrated prior to being stained with Hematoxylin & Eosin (H&E) for histopathological analysis.

Serum transaminases. The levels of circulating ALT and AST were measured in serum samples in a Randox RX Daytona analyser.

Quantification and statistical analysis. For statistical comparison of more than two groups were compared, Kruskal–Wallis test followed by Dunn’s multiple comparisons or two-way analysis of variance was performed using Prism version 7.00 for Windows (GraphPad, La Jolla, CA, USA). Owing to variability in the data, statistical comparison of in vivo work was performed without assumption of normal distribution using Mann–Whitney test. Differences among group mean values were considered significant when the probability value, p, was <0.05, **p < 0.01, ***p < 0.01. Sample size (n) represents a number of biological replicates. No statistical methods were used to predetermine sample size.
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Author contributions

J.J.M., K.M.B., N.B., C.H., and S.A.R. designed the research, analyzed the data, and wrote the paper; J.J.M., C.H., J.A.M., and A.J. conducted experimental design and executed most of the experiments; J.J.M., J.A.M., N.B., M.M.-G., S.A.R., and C.H. carried out in vivo work; L.M., F.D.-P., and K.M.B. provided essential reagents and knowledge.

Competing interests

The authors declare no competing interests.

Additional information

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