SREBP1-induced fatty acid synthesis depletes macrophages antioxidant defences to promote their alternative activation

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Macrophages exhibit a spectrum of activation states ranging from classical to alternative activation. Alternatively, activated macrophages are involved in diverse pathophysiological processes such as confining tissue parasites, improving insulin sensitivity or promoting an immune-tolerant microenvironment that facilitates tumour growth and metastasis. Recently, the metabolic regulation of macrophage function has come into focus as both the classical and alternative activation programmes require specific regulated metabolic reprogramming. While most of the studies regarding immunometabolism have focussed on the catabolic pathways activated to provide energy, little is known about the anabolic pathways mediating macrophage alternative activation. In this study, we show that the anabolic transcription factor sterol regulatory element binding protein 1 (SREBP1) is activated in response to the canonical T helper 2 cell cytokine interleukin-4 to trigger the de novo lipogenesis (DNL) programme, as a necessary step for macrophage alternative activation. Mechanistically, DNL consumes NADPH, partitioning it away from cellular antioxidant defences and raising reactive oxygen species levels. Reactive oxygen species serve as a second messenger, signalling sufficient DNL and promoting macrophage alternative activation. The pathophysiologically relevant mechanism is validated by showing that SREBP1/DNL is essential for macrophage alternative activation in vivo in a helminth infection model.

We analysed RNA-sequencing (RNA-seq) data from human macrophages (GSE117040) and identified SREBP1 as the most upregulated transcription factor in macrophages activated by interleukin-4 (M(IL-4)) compared to those activated by lipopolysaccharide (M(LPS); Fig. 1a). In murine bone marrow-derived macrophages (BMDMs), SREBP1 was among the most activated transcription factor in macrophages activated by interleukin-4 to trigger the de novo lipogenesis (DNL) programme, as a necessary step for macrophage alternative activation. Mechanistically, DNL consumes NADPH, partitioning it away from cellular antioxidant defences and raising reactive oxygen species levels. Reactive oxygen species serve as a second messenger, signalling sufficient DNL and promoting macrophage alternative activation. The pathophysiologically relevant mechanism is validated by showing that SREBP1/DNL is essential for macrophage alternative activation in vivo in a helminth infection model.

Using a publicly available dataset (GSE106706), we observed that induction of SREBP1 target was also dependent on Stat6 (Extended Data Fig. 1c). Altogether, these data demonstrate that SREBP1 activation in alternatively activated macrophages requires both major IL-4-signalling pathways.

SIRT1 and AMPK reduce SREBP1 activity in other models. While not affecting Fasn expression, SIRT1 activation (SIRT1 activator II) slightly increased the expression of Scd2, without affecting M(IL-4) activation (Extended Data Fig. 2a). SIRT1 inhibition (EX-527) did not affect the expression of the SREBP1 target genes Fasn and Scd2, but reduced M(IL-4) activation (Extended Data Fig. 2b), likely in a SREBP1-independent fashion. Inhibition of AMPK activation using compound C had no effect on Fasn and Scd2 expression but induced the expression of the alternative activation marker Retnla while reducing Mgl2 expression (Extended Data Fig. 2c). AMPK activation, using AICAR, only reduced the expression of the SREBP1 target genes Fasn and Scd2 at high concentration. As previously described, AICAR reduced M(IL-4) activation (Extended Data Fig. 2d). Altogether, these data show that AMPK and SIRT1 do not regulate SREBP1 activation in M(IL-4) cells.

To address the specificity of IL-4 activating SREBP1, we stimulated BMDMs with two other macrophage polarizing agents, LPS and dexamethasone. Both LPS and dexamethasone failed to upregulate SREBP1 target genes (Fig. 1c), demonstrating the specificity of IL-4 to activate SREBP1 in macrophages. To confirm that the induction of the SREBP target genes required SREBP activation, we crossed Lyz2Cre+ mice with SREBP cleavage-activating protein (SCAP)fl/fl (M-SCAP-knockout (KO)) mice and cells to disable SREBP genetically. As hypothesized, the induction of SREBP1 target genes in response to IL-4 was reduced in SCAP-KO macrophages (Fig. 1f). Furthermore, pharmacological inhibition of SREBP activation (using 25-hydroxycholesterol (25HC), an inhibitor of SCAP transport), or deletion of SREBP1c, blocked IL-4-induced expression of Fasn and Scd1 (Extended Data Fig. 2e, f). Next, we confirmed IL-4 activated SREBP1 in vivo using IL-4 complex (IL-4c; Fig. 1g), which increased the expression of SREBP1 target genes (Fig. 1h) in...
peritoneal macrophages. Altogether, these data demonstrate that IL-4 is an activator of SREBP1 in macrophages.

While IL-4 strongly activated SREBP1, whether SREBP1 was required for alternative macrophage activation remained unknown. When stimulated with IL-4, BMDMs lacking SCAP had impaired alternative activation compared to wild type (WT; Fig. 2a,b and Extended Data Fig. 3a); however, the inhibition of pro-inflammatory markers was not affected (Extended Data Fig. 3b), indicating that the phenotype was not secondary to a failure to inhibit classical activation. Gene enrichment analysis confirmed the inhibition of alternative activation in SCAP-KO BMDMs compared to controls (Fig. 2c). The dependency of M(IL-4) polarization on SREBP activation was further confirmed pharmacologically using 25HC (Extended Data Fig. 3c–e). Both SREBP1 and SREBP2 are inhibited by 25HC and by SCAP deletion, so we next determined the importance of the isoforms. Deletion of SREBP1c was sufficient to prevent IL-4-induced alternative activation (Extended Data Fig. 3f). We further confirmed that SREBP1 but not SREBP2 was required for macrophage alternative activation in RAW264.7 cells (Extended Data Fig. 3g).

We next assessed the functional relevance of SREBP1 activation in alternative macrophage activation using a pathophysiological infection model, the parasitic helminth *Nippostrongylus brasilien-
sis*. Infection with *N. brasilienensis* triggers a type 2 immune response characterized by type 2 innate lymphoid cells, T helper 2 cells, eosino-
phils and alternatively activated macrophages in the lungs17,18. We infected WT or M-SCAP-KO mice with *N. brasilienensis* and assessed the lungs at 5 and 7 d after inoculation (Fig. 2d). As expected, *N. brasilienensis* infection led to the recruitment of eosinophils, macro-
phages and neutrophils in the lungs (Fig. 2e and Extended Data Fig. 4a and b). Strikingly, M-SCAP-KO mice showed both decreased alveolar macrophage number and alternative activation (Fig. 2e–g and Extended Data Fig. 4c), while interstitial macrophages were not affected (Extended Data Fig. 4d–f). By contrast, the levels of neutro-
phils and eosinophils in the lungs were not altered (Extended Data Fig. 4a,b). We confirmed by histology that the neutrophil score in both alveolar and interstitial spaces was unchanged between genotypes (Extended Data Fig. 4g,h). Alternatively activated alveolar macrophages are essential in limiting tissue damage, notably in the lungs of mice infected with *N. brasilienensis*19–21. M-SCAP-KO mice had an increased red blood cell count in the bronchoalveolar lavage and a trend towards increased alveolar proteinaceous debris compared to controls (Fig. 2h–j). Notably, the alveolar proteinaceous debris score inversely correlated with alveolar macrophage number 5 d after inoculation (Extended Data Fig. 4i). M-SCAP-KO mice also displayed a high number of adult worms in their gut compared to controls at day 5 (Fig. 2k). The worm phenotype could be secondary to the reduced alveolar macrophages number (Fig. 2e–g), as alveolar macrophages kill *N. brasilienensis* larvae in the lung, limiting their arrival in the gut22. Overall, these data demonstrate the pathophysiological relevance of SREBP1 in macrophages.

Our data indicated that SREBP1 was required for macrophage alternative activation, but the underlying mechanism remained unknown. We investigated how the loss of SREBP1 prevented alternative polarization. SREBP1 is a well-established regulator of de novo lipid synthesis23. Accordingly, RNA-seq gene enrichment analysis confirmed that pathways associated with DNL were upregulated in response to IL-4 and blunted in SCAP-KO macrophages (Extended Data Fig. 5a, b). Fatty acid synthase (FASN) protein level was strongly induced by IL-4 in vivo and in vitro in WT but not SCAP-KO cells (Fig. 3a,b). Lipid biosynthesis was substantially induced in IL-4-stimulated macrophages (Fig. 3c) and SCAP-KO cells had reduced lipid synthesis, showing that a loss of SREBP1 activity blunted IL-4-induced DNL (Fig. 3d). While IL-4 induced lipogenesis, LPS-stimulated macrophages only displayed a transient increase in DNL 6 h after stimulation (Extended Data Fig. 5c), showing that sustained lipogenesis is a specific feature of M(IL-4) cells. We next determined the synthesis rate of palmitate. Under control conditions, BMDMs synthesized 0.23 nmol h$^{-1}$ per 10$^5$ cells (1.5% per hour) of palmitate, compared to 0.48 nmol h$^{-1}$ per 10$^5$ cells (2.8% per hour) in IL-4-treated cells (Fig. 3e and Extended Data Fig. 5d). This result confirmed the strong upregulation of DNL by IL-4 and demonstrated that fatty acid synthesis is a significant anabolic process in macrophages.

In line with an accumulation of DNL products, M(IL-4) cells showed an increased level of palmitic (C16:0), palmitoleic (C16:1n7), oleic (C18:1n9) and vaccenic (C18:1n7) acids (Fig. 3f). Overall, IL-4 increased the fatty acid content of the cells by 8.3 nmol per 10$^5$ cells (Fig. 3g), while palmitic acid uptake remained unchanged (Extended Data Fig. 5e). Conversely, SCAP-KO cells exhibited a reduction in DNL-derived fatty acids compared to WT cells (Extended Data Fig. 5f,g), which was not compensated for by increased palmitate uptake (Extended Data Fig. 5h).

To study the role of DNL on M(IL-4) activation, we used two selective FASN inhibitors, C75 and cerulenin. We showed that both inhibitors decreased IL-4-induced lipid synthesis in a dose depen-
dent manner (Extended Data Fig. 6a) and prevented macrophage alternative activation (Fig. 3h,i and Extended Data Fig. 6b–e). However, as for SCAP-KO macrophages, FASN inhibition did not alter the capacity of IL-4 to reduce the expression of the pro-inflammatory cytokines Tnf and Il1b (Extended Data Fig. 6f).

Because SREBPs also regulate cholesterol biosynthesis23, we investigated whether this process was also required for macrophages alternative activation. Inhibition of HMG-CoA reductase with...
simvastatin did not alter macrophage alternative activation, despite a compensatory induction of SREBP2-target genes (Extended Data Fig. 6g,h). In response to LPS, cholesterol supplementation rescues inflammatory responses in FASN-deficient macrophages\(^1\). However, in our model, cholesterol supplementation did not restore the defective alternative activation of SCAP-KO or C75-treated...
macrophages (Extended Data Fig. 7). Given that FASN inhibition with C75 also alters acetoacetate production, we complemented C75-treated macrophages with HMG-CoA to provide substrates to the mevalonate pathway. However, HMG-CoA supplementation failed to rescue the defective alternative activation of C75-treated cells (Extended Data Fig. 6i), further demonstrating that cholesterol biosynthesis and the mevalonate pathway are dispensable for macrophage alternative activation.

We then attempted to rescue the DNL defects by supplementing C75-treated or SCAP-KO cells with fatty acids. We used either 10µM or 50 µM of palmitic acid (PA) or oleic acid (OA), corresponding to the addition of either 20 or 100 nmol per 10⁶ cells for 24 h. Neither PA nor OA significantly reduced Fasn expression at these doses, but OA reduced Scd2 expression (Extended Data Fig. 7). However, neither palmitate nor oleate supplementation restored the alternative activation defects caused by SCAP-KO or FASN inhibition (Fig. 3j,k).
Fig. 2 | Inhibition of SREBP1 activation impairs macrophages alternative activation and immune response to helminths. a, Alternative activation assessed by the co-expression of RELMα and CD206 by flow cytometry of WT and SCAP-KO BMDMs in response to IL-4. Quantification of the number of M(IL-4) macrophages is presented as the mean ± s.e.m. in b. Data are from n = 8 biological replicates from two independent experiments. c, Gene enrichment analysis of the pathways associated with response to IL-4 and innate immune response of the interaction effect of IL-4 in WT and SCAP-KO BMDMs. Data are from n = 6 biological replicates from two independent experiments per group. d, Experimental design for N. brasiliensis infection. BAL, bronchoalveolar lavage. e, Macrophage number in the lungs of naïve mice or WT and M-SCAP-KO mice 5 and 7 d after N. brasiliensis inoculation. Data are expressed as the mean ± s.e.m. f, Percentage of alternatively activated alveolar macrophages assessed by the co-expression of RELMαs.e.m. expressed as the mean. Red blood cell counts in the BAL of WT and M-SCAP-KO naïve mice or 5 and 7 d after N. brasiliensis inoculation. Data are expressed as the mean ± s.e.m. h, Red blood cell counts in the BAL of WT and M-SCAP-KO naïve mice or 5 and 7 d after N. brasiliensis inoculation. Data are expressed as the mean ± s.e.m. i, Representative pictures of H&E staining of lung sections from four WT and four M-SCAP-KO mice 5 d after N. brasiliensis inoculation. Black arrowheads indicate alveolar proteinaceous debris. Scale bars, 100 μm. j, Histological score of alveolar proteinaceous debris in WT and M-SCAP-KO naïve mice or 5 and 7 d after N. brasiliensis inoculation. Data are expressed as the mean ± s.e.m. k, Adult worm count in the gut of WT and SCAP-KO naïve mice or 5 and 7 d after N. brasiliensis inoculation. Data are expressed as the mean ± s.e.m. For the N. brasiliensis infection experiment, data from n = 6 (WT, day (D)0), n = 7 (KO D0), n = 11 (WT D5) and n = 12 (WT D7 and KO D5 and D7) mice from two independent experiments are shown. Data were analysed using a two-way ANOVA followed by Sidak post hoc test for comparison between genotypes in control or IL-4-treated cells or at different days after N. brasiliensis inoculation.

and Extended Data Fig. 7), indicating that the lipids produced by DNL were not the signal regulating alternative activation.

We then investigated the mechanisms by which macrophages sense DNL. We noted that pathways associated with redox homeostasis and oxidative processes were upregulated in response to IL-4 in WT cells (Extended Data Fig. 8a) and failed to be induced by IL-4 in SCAP-KO BMDMs (Fig. 4a). Given that reactive oxygen species (ROS) would be a plausible secondary messenger for macrophage alternative activation29–32, we assessed ROS levels in WT and SCAP-KO cells and showed that IL-4 induced ROS in WT but not in SCAP-KO BMDMs (Fig. 4b). Similarly, blocking SREBP activation using 25HC treatment also reduced ROS levels after IL-4 stimulation in BMDMs (Extended Data Fig. 8b). The induction of cellular ROS levels by IL-4 was also blunted when FASN was inhibited with C75 or cerulenin, indicating that the loss of FASN activity mediated the ROS depletion observed when SREBP1 was inactivated (Fig. 4c and Extended Data Fig. 8c).

We next determined the source of ROS in IL-4-treated macrophages. Inhibition of canonical ROS producers, such as NADPH oxidases, nitric oxide synthase and xanthine oxidase did not affect ROS levels in M(IL-4) cells, but reduced LPS-induced ROS (Extended Data Fig. 8d). Conversely, we confirmed the findings of previous studies that reported increased mitochondrial ROS production in response to IL-4 (Extended Data Fig. 8e)24,33.

Previous studies suggested that fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) are required for macrophage alternative activation24,34, and that OXPHOS is a potential source of oxidative phosphorylation (OXPHOS) are required for macrophage alternative activation23–27, we assessed ROS levels in WT and SCAP-KO cells and showed that IL-4 induced ROS in WT but not in SCAP-KO BMDMs (Fig. 4b). Similarly, blocking SREBP activation using 25HC treatment also reduced ROS levels after IL-4 stimulation in BMDMs (Extended Data Fig. 8b). The induction of cellular ROS levels by IL-4 was also blunted when FASN was inhibited with C75 or cerulenin, indicating that the loss of FASN activity mediated the ROS depletion observed when SREBP1 was inactivated (Fig. 4c and Extended Data Fig. 8c).

Seeking an alternative explanation for the reduced ROS levels observed when FASN activity was decreased, we focused on the bioenergetic requirements of DNL. FASN uses NADPH, a cofactor also required for the replenishment of the glutathione (GSH) antioxidant defences. To determine if DNL consumed sufficient NADPH in macrophages to dysregulate the GSH antioxidant system, we used [3–H]glucose to measure NADPH consumption by palmitic acid synthesis35.

Palmitate synthesis consumed ~3.3 nmol h−1 of NADPH per 106 cells under basal conditions, rising to ~5.4 nmol h−1 of NADPH per 106 cells in alternatively activated macrophages and NADPH consumption was reduced by FASN inhibition (Fig. 4d). Mimicking the reduced NADPH consumption due to DNL inhibition, SCAP-KO

Fig. 3 | Limiting SREBP1-induced lipid synthesis reduces macrophages alternative activation without altering macrophages lipid composition. a, In vivo induction of FASN protein expression in MACS-sorted peritoneal macrophages (F4/80+) from mice injected with IL-4c or pBS. β-actin was used as the loading control. b, Protein expression of FASN in WT and SCAP-KO BMDMs in response to IL-4. Representative western blot analysis of FASN protein expression of FASN in WT and SCAP-KO BMDMs treated with DMSO or C75 (10 μM) or oleate (10 μM). The quantification of the number of alternatively activated macrophages from n = 4 biological replicates is presented as the mean ± s.e.m. in k. Data were analysed using two-tailed Student’s t-test (Fig. 3c,e) or a two-way ANOVA followed by a Sidak (Fig. 3d,f,g), Dunnett (Fig. 3i) or Tukey (Fig. 3k) post hoc test.
BMDMs had an increased NADPH/NADP⁺ ratio following IL-4 treatment relative to controls (Fig. 4e). In support of the concept that fatty acid synthesis and antioxidant defences compete for NADPH in alternatively activated macrophages, reduced GSH levels were decreased following IL-4 stimulation and increased in both SCAP-KO-treated cells and C75-treated cells (Fig. 4f and Extended Data Fig. 8p). Importantly, GSH levels and the NADPH consumption rate due to palmitate synthesis were both within the 2–4 nmol per 10⁶ cells range. As the half-life of GSH in macrophages has been reported to be 1.9 h⁰, consumption of NADPH by DNL
Fig. 4 | SREBP1-dependent lipid synthesis increases NADPH utilization to reduce antioxidant defences and permit macrophage alternative activation.

**a.** Gene enrichment analysis of the pathways associated with oxidoreduction processes of the interaction effect of IL-4 in WT and SCAP-KO BMDMs. Data of \(n=6\) biological replicates from two independent experiments per group. **b.** ROS levels in WT and SCAP-KO BMDMs in CTR or IL-4-treated cells. ROS were quantified by the median fluorescence intensity (MFI) of CM-H2DCFDA by flow cytometry. Data are the mean ± s.e.m. of \(n=4\) biological replicates. **c.** ROS levels in DMSO-treated or C75-treated \((10 \mu M)\) BMDMs in CTR or IL-4-treated cells. ROS were quantified by the MFI of CM-H2DCFDA by flow cytometry. Data are the mean ± s.e.m. of \(n=11\) biological replicates from three independent experiments. **d.** NADPH consumption rate by palmitate synthesis in BMDMs treated with DMSO or cerulenin \((1 \mu g \text{ ml}^{-1})\) in response to IL-4. Data are presented as the mean ± s.e.m. from \(n=4\) (DMSO) and \(n=3\) (cerulenin) biological replicates. **e.** NADPH/NADP⁺ ratio in WT and SCAP-KO BMDMs in response to IL-4. Data are presented as the mean ± s.e.m. from \(n=8\) biological replicates from two independent experiments. a.u., arbitrary units. **f.** Reduced GSH levels in WT and SCAP-KO BMDMs in response to IL-4. Data are the mean ± s.e.m. of \(n=4\) biological replicates. **g.** \(H_2O_2\)-induced cell death \((100 \mu M, 24 \text{ h})\) challenge in WT and SCAP-KO BMDMs determined by flow cytometry using a live/dead dye. The quantification of dead cells (%) is shown in the right graph. Data are the mean ± s.e.m. of \(n=4\) biological replicates. **h.** Cellular ROS levels in CTR or IL-4-stimulated WT and SCAP-KO BMDMs pretreated with NAC. ROS were quantified by the fluorescence ratio of CM-H2DCFDA over DNA (Hoechst). Data are the mean ± s.e.m. of \(n=8\) biological replicates from two independent experiments. **i.** Alternative activation (AA) assessed by the co-expression of RELMα and CD206 by flow cytometry of WT and SCAP-KO BMDMs in response to IL-4 and NAC. The quantification of the number of M(IL-4) macrophages as the mean ± s.e.m. of \(n=11\) (WT) and \(n=13\) (KO) biological replicates from three independent experiments is presented in **j.** Statistical analysis of the RNA-seq data is detailed in the Methods. Data were analysed using a two-way ANOVA followed by Sidak post hoc test (Fig. 4b–j).
could plausibly regulate GSH levels. Finally, SCAP-deficient macrophages were protected against H₂O₂-induced cell death (Fig. 4g), further indicating they possessed increased antioxidant defences. Altogether, our results demonstrate that, in macrophages, IL-4-induced DNL can consume enough NADPH to impact on antioxidant defences, thus increasing ROS levels.

We confirmed the relevance of IL-4-induced ROS for alternatively activated macrophages by treating BMDMs with N-acetylcysteine (NAC) to artificially replenish the GSH pool, independently of NADPH levels. NAC treatment blunted the accumulation of ROS following IL-4 stimulation (Extended Data Fig. 9a) and impaired the M(IL-4) polarization of the BMDMs (Extended Data Fig. 9b–d). As SCAP-KO BMDMs already had increased antioxidant defences due to their lack of DNL, treating them with NAC did not reduce ROS levels (Fig. 4h) or alternative activation any further (Fig. 4i,j) in M(IL-4). Overall, our data support a mechanism whereby DNL increases ROS levels to enable M(IL-4) activation.

This study has identified the SREBP1–DNL anabolic network as a major downstream effector of IL-4 signalling in macrophages (Extended Data Fig. 10) and the pathophysiological relevance of SREBP1 activation in macrophages for the immune response in vivo using a parasite infection model. The activation of SREBP1 in response to IL-4 is conserved in mouse and human macrophages, suggesting targeting this pathway may have therapeutic value. Our data show a dramatic reduction in DNL 24 h after LPS stimulation, indicating that lipid accumulation in pro-inflammatory macrophages is likely to come from uptake/or reduced FAO rather than increased fatty acid synthesis.

In M(IL-4)-4 cells, we show the high rate of lipid synthesis in macrophages consumes substantial quantities of NADPH, limiting its availability for antioxidant defences, leading to ROS accumulation. ROS acts as a necessary second messenger linking SREBP1 activation to macrophage alternative activation. While ROS is already established as a regulator of macrophage alternative polarization, a limitation of our study is the absence of any rescue of alternative activation due to inhibition of DNL by pharmacological ROS-inducing agents. This result highlights the complexity of oxidative and reductive stresses and potentially indicates that the specific source of ROS production may be significant, and the link between DNL and ROS production, notably in vivo, requires further investigation.

While the importance of ROS levels has been established, the role of the actual lipids synthesized in response to IL-4 remains to be elucidated. Of potential relevance, while in vitro IL-4 does not alter macrophage number, IL-4 is a potent driver of macrophage proliferation in vivo, a process that requires the biosynthesis of new lipids to supply the formation of new cell membranes. Therefore, it could be argued that DNL could support new membrane synthesis required for IL-4-induced macrophage proliferation. In this context, ROS may serve as a proxy of enough anabolic activity to engage in a demanding proliferative alternative activation. Notably, the lower number of alveolar macrophages in our helminth infection model supports this hypothesis. In support of the concept that anabolism and macrophage alternative activation are intrinsically linked, inhibition of the non-oxidative arm of the pentose phosphate pathway, which is required for nucleotide synthesis, also impairs macrophage alternative activation. Moreover, in vitro inhibition of Myc, a major transcription factor governing cellular proliferation, also impedes macrophage alternative activation. Altogether, these studies indicate the relevance of anabolic pathways for proliferation ability and alternative activation in macrophages. Further studies should address the interdependence of these pathways in disease models involving macrophage alternative activation and proliferation, such as cancer and parasitic infections.

**Methods**

**Animal models.** All animal work was carried out in the Disease Model Core or the Medical Research Council (MRC) Laboratory of Molecular Biology (LMB) ARES unit.

Animals were housed in a temperature-controlled room (22°C) with a 12-h light/dark cycle with 55% relative humidity (DMLC) and 45–65% relative humidity (MRC-LMB ARES). Food and water were available ad libitum. This research was regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body and the MRC ARES animal facility, under pathogen-free conditions and housing according to UK Home Office guidelines.

For macrophage-specific gene deletion, we used a mouse model expressing Cre recombinase from bacteriophage P1 under Lyz2 promoter (Lyz2-Cre). Lyz2-Cre mice were generated by replacing a single allele of the Lyz2 gene with Cre recombinase coding sequence as described and was gifted to us on a mixed C57BL/6J, 129/Sv background by S. Jackowski. The Lyz2-Cre line was backcrossed to the C57BL/6J background using marker-assisted accelerated backcrossing (MAX-BAX, Charles River) technology until single-nucleotide polymorphism genotyping confirmed >99% background purity.

The macrophage-specific SCAP ablation model was generated by crossing the B6;129-Sca1tm1Myj/J mice obtained from Jackson Laboratories (004162) with the Lyz2-Cre mouse model. SCAP macrophage-specific KO mice were produced by crossing Lyz2-Cre mice with Lyz2C战略合作 animals on a floxed/floxed background, yielding a 1:1 Mendelian ratio of control (floxed/floxed Lyz2C战略合作) to KO (floxed/floxed Lyz2C战略合作) offspring.

SREBP1C-KO mice were previously described. Studies were conducted in 2- to 4-month-old male mice using littermate controls. Details of the mouse models are provided in Supplementary Table 2.

**Bone marrow-derived macrophage cell culture.** Six- to sixteen-week-old male C57BL/6J mice, obtained from Charles River, Lyz2C战略合作 or Lyz2C战略合作 SCA1tm1Myj/J, or SREBP1C-KO mice were culled and the bone marrow was flushed from the femur and tibia in RPMI 1640. Total bone marrow cells were passed into a 100-μm cell strainer and counted using a Countess II automated cell counter (Thermo Fisher). Cells were spun (400g for 5 min), resuspended in BMDM culture medium (RPMI 1640 supplemented with 20% of 19:29- conditioned cell medium, 10% heat-inactivated fetal bovine serum (FCS) and 1% penicillin–streptomycin). Total bone marrow cells were seeded in 10-cm non-culture-treated plates (Falcon) at a density of 5 × 10⁶ cells per plate per 10 ml of macrophage differentiation medium, and cultured for 7 d at 37°C in 5% CO₂. On day 5 of differentiation, the medium was removed and replaced with 10 ml of fresh BMDM culture medium. On day 7, BMDMs were detached using ice-cold PBS-ETDA (1 mM), spun (400g...
Isolation of cells from mice. Animal-free recombinant murine IL-4 (AF-214-14, subcutaneously with 400 viable third-stage Nippostrongylus brasiliensis through Wistar strain rats. Lyz2 SCAPfl/fl mice were inoculated or Lyz2Cre/−/ mice were infected.

Flow cytometry staining and analysis. For the lungs, the gating strategy for the lung immune cells was as described below.

Magnetic-activated cell sorting. PECs were resuspended in MACS buffer (PBS, 2 mM EDTA (sterile), 0.5% BSA) and incubated with biotin-conjugated anti-IFN-γ antibody (Clone REA126, Miltenyi Biotech, 130-101-893) for 10 min at 4 °C. Then, PECs were incubated with Streptavidin Microbeads (Miltenyi Biotech, 130-048-101) for 15 min at 4 °C. Finally, the F4/80− fraction was isolated using MACS LS columns according to the manufacturer’s instructions (Miltenyi Biotech). Samples were further processed for immunoblotting or gene expression analysis as described below.

For each mouse, a blinded histopathologist scored 20 randomly chosen fields for the presence of neutrophils in the alveolar and interstitial spaces and the presence of proteinaceous debris according to the recommendations of the American Thoracic Society. The scoring data are expressed as the sum of the score of the 20 fields.

De novo lipid synthesis assay. BMDMs were seeded in a non-tissue-culture–treated 24-well plate at a density of 250,000 cells per well and stimulated as described in the figure legends. Then, incorporation of 14C-sodium acetate into lipids was assessed over a 2-h period. After medium removal, cells were detached with ice-cold PBS-EDTA. Cell suspensions were transferred into glass vials, counted, spun and the supernatant discarded. The cell pellets were snap frozen in dry ice before lipid extraction. Total lipids from cells were extracted using the protocol described below, and radioactivity was measured using liquid scintillation counter.

Fatty acid oxidation assay. BMDMs were seeded in a 24-well plate at a density of 250,000 cells per well and stimulated as described in the figure legends. At 24 h after IL-4 stimulation, cells were washed with PBS and incubated in the presence of 0.5 ml of fatty FAO medium (RPMI containing 12.5 mM HEPES, 1 mM t-carnitine, 10% HI-FBS and 0.2 mgC−1 [1-14C]oleate (NEC317250UC, Perkin Elmer)). Plates were then immediately sealed with parafilm M and placed at 37 °C for 3 h. Meanwhile, CO2 traps were prepared by adding 200 μl of concentrated HCl into the headspace of Eppendorf tubes containing paper discs, wove with 20 mg of 1M NaOH, in the inner side of their lids. Once the FAO reaction finished, 400 μl of medium from BMDMs was transferred into the CO2 traps, lids were immediately closed and tubes were incubated for 1 h at room temperature, allowing CO2 to escape the medium and react with NaOH in the paper disc. Paper discs were then transferred to scintillation vials containing 5 ml of Hionic-Fluor scintillation liquid, and radioactivity was measured using liquid scintillation counter.

Lipid extraction. One million cells were seeded in non-tissue culture plates and detached with ice-cold PBS-EDTA. Cell suspensions were transferred into glass vials, counted, spun and the supernatant discarded. The cell pellets were snap frozen in dry ice before lipid extraction. Total lipids were extracted using the protein precipitation method2. Glass pipettes and vials were used throughout the procedure to avoid plastic-bound lipid contamination. Cell pellets were resuspended in 1 ml of HPLC-grade chloroform:methanol at 2:1 vol/vol mixture.

Flow cytometry and Western blotting analysis. For BMDMs, cells were seeded in a non-tissue culture–treated plate and detached using ice-cold PBS-EDTA (1 mM). After collection, lung digest or PECs or BMDMs were kept in FACS buffer (PBS, 1 mM EDTA and 3% HI-FBS) on ice. Non-specific binding was blocked with 5% of 11b11. Each mouse was injected intraperitoneally with 200 μg of 11b11 glucose) supplemented with 10% HI-FBS, 100 U ml−1 penicillin–streptomycin and 2 mM L-glutamine (Sigma) for a density of 250,000 cells per 50 ml of medium for each T175 tissue culture flask. Medium was collected after 1 week of culture, and then 50 ml of fresh DMEM supplemented with 10% HI-FBS, 100 U ml−1 penicillin–streptomycin and 2 mM L-glutamine was added onto cells and collected 1 week later. BMDMs were then counted using a Countess II automated cell counter and cell pellets were snap frozen in dry ice before lipid extraction. Total lipids from cells were extracted using the protocol described below.
Deuterated tridecanoic acid (DLM-1392-PK, Cambridge Isotopes) was included in extraction mixture as the internal standard. Samples were homogenized by vortexing for 15 s. Then, 400 µl of HPLC-grade acetone was added to each sample before vortexing for 2 min and centrifuging at 4,000g for 30 min. The single layer of supernatant was pipetted into a separate glass vial while being careful not to break up the solid pellet at the bottom of the tube. Collected lipid fractions were dried under nitrogen stream.

**Quantitative analysis of fatty acid methyl esters.** To derive free fatty acids and esterified fatty acids from complex lipids into FAMEs, 750 µl of HPLC-grade chloroform: methanol at a 1:3 vol/vol solution was added to previously dried lipids in 7-ml glass vials. In total, 90 µl of 13% boron trifluoride in methanol (134821, Sigma) was then added into each vial. Vials were sealed and incubated in an oven at 80 °C for 90 min to hydrolyse fatty acid–glycerol and fatty acid–cholesterol ester bonds and form FAMEs. Samples were allowed to cool, and 1 ml of HPLC-grade n-hexane and 500 µl of HPLC-grade water were added. Samples were briefly vortexed and centrifuged at 2,000g. The upper organic layer was transferred into 2-ml gas chromatography glass vials and dried under nitrogen stream.

Gas chromatography–mass spectrometry was performed with the Agilent 7890B gas chromatography system linked to an Agilent 5977A mass spectrometer using an AS5000 auto sampler, and data were acquired using MassHunter Workstation Software. A TR-FAME column (length of 30 m, internal diameter of 0.25 mm, film size of 0.25 µm; 260M142P, Thermo Fisher Scientific) was used with helium as the carrier gas. Inlet temperature was set at 230 °C. Dried FAME samples were reconstituted in 200µl HPLC-grade n-hexane. A total of 1 µl of this solution was injected for analysis. The oven programme used for separation was as follows: 100 °C hold for 2 min, ramp at 25 °C per min to 150 °C, ramp at 2.5 °C per min to 162 °C and hold for 3.8 min, ramp at 4.5 °C per min to 173 °C and hold for 5 min, ramp at 5 °C per min to 210 °C, ramp at 40 °C per min to 230 °C and hold for 0.5 min. The carrier gas flow was set to a constant rate of 1.5 ml min⁻¹. If the height of any FAME peaks exceeded 10⁸ units, the sample was re-injected with a 10:1–100:1 split ratio.

We determined a theoretical distribution for a newly synthesized molecule that was dependent on the precursor labelling pool, whereby p was the fraction of deuterated NADPH. Then, we calculated the isotopomer pattern of palmitate caused by the presence of additional ²H atoms in the molecule. The number of available sites in palmitate that a deuterium could be incorporated was assumed to be 14 based on previous publications. We extracted the M⁺ ion of palmitate methyl esters (m/z 270–275). From these we calculated the fractional concentration of each ion. All equations below were derived with the Solver function of Excel to minimize the sum of squares of M₀obs–M₀obs by permitting the values of p and f to be adjusted. This enabled us to calculate the fractional synthesis rate for palmitate.

**NADPH consumption rate** was determined using the quantification of palmitate quantity within the cell and the fraction synthesis rate (f) determined as described above using [3-²H]glucose tracing and the number of NADPH molecules required to make one molecule of palmitate (14):

\[
\text{NADPH consumption rate} = \text{palmitate quantity (nmol per 10⁶ cells)} 
\times (\frac{p}{f \text{(palmitate)}}) \times 14
\]

**Palmitate synthesis and uptake rate using deuterated water and stably labelled palmitate.** One million cells were seeded in non-tissue culture plates and stimulated as described above. Two hours before harvesting, the medium was replaced with IMDM culture medium supplemented with 8% deuterated water (151882, Sigma) and 10 µM [³H]palmitate (Cambridge Isotope Laboratories, CLM-409-PK). The cells were moved into an incubator filled with 8% deuterated water to maintain the concentration of deuterated water in the wells at 8%. Cells were detached with ice-cold PBS-EDTA and lipids extracted and derivatized as FAME, as detailed above.

We determined a theoretical distribution for a newly synthesized molecule that was dependent on the precursor labelling pool, whereby p was the fraction of deuterated NADPH. Then, we calculated the isotopomer pattern of palmitate caused by the presence of additional ²H atoms in the molecule. The number of available sites in palmitate that a deuterium could be incorporated was assumed to be 14 based on previous publications. We extracted the 14/14/13 ions of palmitate methyl esters (m/z 270–275). From these we calculated the fractional concentration of each ion. All equations below were derived with the Solver function of Excel to minimize the sum of squares of M₀obs–M₀obs by permitting the values of p and f to be adjusted. This enabled us to calculate the fractional synthesis rate for palmitate.

**NADPH consumption rate** was determined using the quantification of palmitate quantity within the cell and the fraction synthesis rate (f) determined as described above using [3-²H]glucose tracing and the number of NADPH molecules required to make one molecule of palmitate (14):

\[
\text{NADPH consumption rate} = \text{palmitate quantity (nmol per 10⁶ cells)} 
\times (\frac{p}{f \text{(palmitate)}}) \times 14
\]
We calculated M0obs–M0obs through to M5obs–M5obs and calculated the sum of squares for these six equations. We used the GRG Non-Linear Engine of the Solver function of Excel to minimize the sum of squares of M0obs–M0obs, M1obs–M1obs, M2obs–M2obs, M3obs–M3obs, M4obs–M4obs, and M5obs–M5obs by changing the values of N and f.

While in vivo the N for palmitate is established to be 22, there is considerable evidence that N varies in vitro. Given p was known and experimentally fixed at 8%, we therefore determined Nb by a trial and error and selected an N value that gave a p of 8%. This enabled us to calculate the fractional synthesis rate for palmitate and the N number.

The rate of synthesis of palmitate from DNL was determined using the palmitate quantity within the cell and the fraction synthesis rate (f) as determined above:

De novo palmitate synthesis rate per hour = palmitate quantity (nmol per 10⁶ cells) × f/palmitate/2

We divided by 2 as we labelled for 2h.

Exogenous palmitate uptake was measured using the sum of the integration of + 16 peaks of palmitate, palmitoleate, stearate, oleate and vaccinate and expressed as nmol per hour per 10⁶ cells.

Oxygen-consumption rate measurement. BMDMs were plated in XF-96 culture plates at a density of 50,000 cells per well, treated or not with 250 µM lipoic acid and polarized into M(IL-4) macrophages with IL-4 (10 ng ml⁻¹) for 4 h. Oxygen-consumption rate measurement was performed using an XF-96 Extracellular Flux Analyser (Seahorse Bioscience). To assess intrinsic mitochondrial respiration, three different oxygen-consumption rate measurements were obtained under basal conditions and after the sequential addition of 1 µM oligomycin, to inhibit mitochondrial ATP synthase; 1.5 µM rotenone plus 1 µM antimycin A, which inhibit the electron-transport chain. All drugs were purchased from Sigma.

Reactive oxygen species measurement. For the measurement of intracellular and mitochondrial ROS production, BMDMs were seeded at 50,000 cells per well in a black 96-well plate and polarized and/or treated as described above. BMDMs were incubated in RPMI 1640 at 37 °C in 5% CO₂ with 10 µM CM-H2DCFDA (Thermo Fisher Scientific), as cytosolic ROS indicator, for 15 or 30 min. Cells were then incubated for 1 h in a non-CO₂ incubator, following the manufacturer's instructions.

Statistical analysis of the RNA-seq data was performed in R (build 3.4.1 Single. Ensembl IDs were converted to gene symbols using the ensembl ‘mmusculus_gene_ensemble’ dataset’. Duplicated genes counts were summed and genes that had an average number of counts of less than five were filtered out. Counts were log-normalized using Variance Normalization Stabilization (van设有) for each of the two batches individually, and then batch effect correction was applied using the R/Bioconductor package ‘sva’ (sva library’). Principal-component analysis was performed with the pcomp function and cross-correlation was done using Pearson correlation and the complete clustering method on Euclidian distances with heatmap2. Differential expression analysis was performed using limma library’. Contrast matrices were created to compare the effect of IL-4 compared to control (contrast, IL-4 WT − control WT), the KO compared to WT (contrast, control KO − control WT) and the effect of KO on the IL-4 response (contrast, IL-4 KO − IL-4 WT + control WT − control KO).

RT–qPCR was performed in a 13-µl reaction with 5 µl of diluted cDNA, 6.5 µl of 2× TaqMan or SYBR Green reagent (Applied Biosystems), 1.3 µl of 3 mM forward and reverse primer mix (including 1.5 mM of probe for TaqMan reactions) and 0.2 µl of RNase-free water, according to the default manufacturer's protocol (Applied Biosystems). Primer sequences are described in Supplementary Table 3. Reactions were run in duplicate for each sample and quantified using the ABI Prism 7900 sequence detection system (Applied Biosystems). Duplicates were checked for reproducibility, and then averaged; ‘no reverse transcriptase’ controls were included to check for genomic DNA contamination, and ‘no template’ controls were included to check for the formation of primer dimers. Product specificity was determined using a dissociation curve for SYBR green reactions. A standard curve generated from a pool of all cDNA samples was used for quantification. The expression of genes of interest was normalized using the BestKeeper method to the geometric average of three housekeeping genes (18S, 36B and Tbp), and data were expressed as arbitrary units or normalized to the average of the control group. The list of primers is available in Supplementary Table 4.

Library preparation and RNA-seq. A total of 1 µg of total RNA was quality checked (RNA integrity number > 7) using an Agilent Bioanalyzer 2100 system and used to construct barcoded sequencing libraries with the Illumina TruSeq Stranded mRNA Library Prep Kit following the manufacturer's instruction. All the libraries where then multiplexed and sequenced on one lane of Illumina HiSeq 4000 at SEQUO at CRUK Cambridge Institute Genomics Core Facility.

DNAseq data analysis. Sequence reads were mapped onto the GRCm38 genome using TopHat v2.0.11 and then genes were counted using htseq-count V0.6.1p1. Statistical analysis of the RNA-seq data was performed in R (build 3.4.1 Single). Ensembl IDs were converted to gene symbols using the ensembl ‘mmusculus_gene_ensemble’ dataset’. Duplicated genes counts were summed and genes that had an average number of counts of less than five were filtered out. Counts were log-normalized using Variance Normalization Stabilization (van设有) for each of the two batches individually, and then batch effect correction was applied using the R/Bioconductor package ‘sva’ (sva library’). Principal-component analysis was performed with the pcomp function and cross-correlation was done using Pearson correlation and the complete clustering method on Euclidian distances with heatmap2. Differential expression analysis was performed using limma library’. Contrast matrices were created to compare the effect of IL-4 compared to control (contrast, IL-4 WT − control WT), the KO compared to WT (contrast, control KO − control WT) and the specific effect of the KO on the IL-4 response (contrast, IL-4 KO − IL-4 WT + control WT − control KO).

Enrichment analysis was performed using a consensus of multiple enrichment methods, including PANTHER (all available methods in PANTHER) and using miPathway (these make use of a gene-set collection comprising msigdb c2 canonical pathways, Gene Ontology biological processes terms and chemical and genetic perturbations (cgp). Pathway analysis results of the different contrasts are available in Supplementary Table 1. Results are expressed as adjusted P values for ‘distinct’, ‘mixed’ (up or downregulated) and ‘non-directional’. ‘Distinct’ refers to an enrichment accounting for the over-representation of measured genes in a pathway compared to all measured genes in the experiment. A mixed-up enrichment corresponds to an enrichment of only significantly upregulated genes in a pathway with respect to only upregulated genes in the experiment, and vice-versa for mixed-down. In the figures, only the ‘distinct’ and ‘non-directional’ results are presented. The pathway analyses are presented in Supplementary Table 1.

The transcriptional regulators prediction analysis was performed using the upstream regulator function of Ingenuity Pathway Analysis. Analysis was performed to predict for ‘transcription regulator’ and ‘ligand-dependent nuclear receptor’. The data presented in Fig. 1a have been produced with a cut-off of a false discovery rate (FDR) < 0.01 and the human macrophages using the GSE117040 dataset with a cut-off of FDR < 0.05 and log₂FC (M1/M2) > 1.0. Plots were made using ggplot2 library.

Immunoblot analysis. Cells were washed in ice-cold PBS and lysed in RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP-40, pH 7.4) supplemented with complete, Mini, EDTA-free Proud Inhibitor Cocktail (Roche) and 1% sodium deoxycholate. Protein concentration was quantified by DC protein assay (500011, Bio-Rad) and adjusted in RIPA buffer. Lysates were diluted in NuPAGE LDS sample buffer (NP0007, Thermo Fisher Scientific) containing 2.5% 2-mercaptoethanol and boiled at 95°C for 5 min. For the detection of SREBP1, the samples were not boiled but incubated at 37°C for 1 h before loading into the gel. Next, 25-µg protein of each was then separated by electrophoresis using NuPAGE SDS–polyacrylamide gels (Thermo Fisher Scientific) and transferred to nitrocellulose membranes using the iBlot Dry Blotting System (Thermo Covid-19).
Fisher Scientific). Membranes were blocked for 1 h in 3% BSA in Tris-buffered saline containing 0.05% Tween (TBS-T) at room temperature. Membranes were incubated overnight with the following primary antibodies: mouse anti-SREBP1 (ab3259, clone 2A4, Abcam), rabbit anti-fatty acid synthase (3180, clone C20G5, Cell Signaling), mouse anti-β-actin (3700, clone 8H10D10, Cell Signaling), rabbit anti-phospho-AKT Ser473 (4060, clone D9E, Cell Signaling) and rabbit anti-AKT (9272, Cell Signaling). Bound primary antibodies were detected using peroxidase-coupled secondary anti-rabbit (7074, Cell Signaling) or anti-mouse (7076, Cell Signaling) and enhanced chemiluminescence (WBULU0500, Millipore). Images were acquired using the ChemiDoc MP System (Bio-Rad), and bands were quantified using Fiji software (https://imagej.net/software/fiji/). The expression of proteins was normalized to a housekeeping protein (β-actin), and the phosphorylation status was determined by normalizing to a respective total protein. All protein quantification data are expressed as arbitrary units. A detailed list of the antibodies used for this study is presented in Supplementary Table 1.

**NADPH assay.** NADPH concentration was determined using the NADP/NADPH-Glo Assay (Promega), following the manufacturer's instructions. NADP+ levels were determined from the standard curve.

**Glutathione assay.** Reduced GSH concentration was determined using the GSH-Glo Assay (V6911, Promega), according to the manufacturer's instructions.

**Statistical analysis.** RNA-seq statistical analysis is detailed in the RNA-seq methods section. Overall, statistical significance was determined using two-tailed Student’s t-test, and a one-way or two-way ANOVA followed by the appropriate post hoc test as indicated in the figure legends. For the ANOVA, the result of the post hoc test was only considered if the genotype/treatment effect was significant. P < 0.05 was considered as statistically significant and all the data were analysed using GraphPad Prism (version 9.1.0).

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All the data presented in this manuscript are available upon reasonable request to the corresponding authors. Transfer of materials requires materials transfer agreements. The RNA-seq dataset is deposited in the Gene Expression Omnibus under accession number GSE179066. Source data are provided with this paper.

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10. Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

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Extended Data Fig. 1 | IL-4 induced SREBP1 activation is downstream of AKT and STAT6 signalling. a, Protein expression of phosphorylated (Ser473) and total Akt in Vehicle (DMSO) or MK-2206-treated BMM in response to IL-4. Representative picture of n = 4 biological replicates. b, mRNA expression over BK of the SREBP target genes Fasn and Scd2 AKT in Vehicle (DMSO) or MK-2206-treated BMM in response to IL-4. Data are presented as the mean ± SEM of n = 8 biological replicates from 2 independent experiments. c, Gene expression analysis of the SREBP target genes in WT and Stat6<−/> BMM. Data analysed from the publicly available dataset (GSE106706) with FDR<0.05 and Fc>2. Data has been analysed using a 2-way ANOVA followed by Sidak post-hoc test.
Extended Data Fig. 2 | SIRT1 and AMPK are not responsible for SREBP1 activation in response to IL-4. a–d, mRNA expression of the SREBP1 target genes Fasn and Scd2 and of the macrophages alternative activation markers Retnla and Mgl2 in BMMφ treated with SIRT1 activator II (a) or SIRT1 inhibitor (EX-527, b) or the AMPK inhibitor Compound C (c) or the AMPK activator AICAR (d) in response to IL-4. Data are presented as the mean ± SEM of n=4 biological replicates per group. e and f, mRNA expression over BK of the SREBP1 target genes in 25-hydroxycholesterol-treated (25HC, n=8 biological replicates from 2 independent experiments) (e) or SREBP-1c KO (n=3 biological replicates) (f) BMMφ in response to IL-4. Data has been analysed using a 2-way ANOVA followed by a Dunnett (a–d) or Sidak (e and f) post-hoc test.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | SREBP1 but not SREBP2 activation is required for macrophage alternative activation.  
a and b, mRNA expression of Retnla and Mgl1 (a) and Tnf and Il1b (b) in WT and SCAP-KO BMMΦ, 4h and 24h post IL-4 stimulation. mRNA expression over BK as mean ± SEM of n=8 biological replicates from 2 independent experiments. c, Alternative activation of Vehicle (EtOH) or 25-hydroxycholesterol (25HC)-treated BMMΦ in response to IL-4. Alternative activation was assessed by the expression of RELMα and CD206 by flow cytometry. The quantification of the number of alternatively activated macrophages is presented as mean ± SEM in d. Data of n=4 biological replicates per group. e, Expression of the macrophage alternative activation markers Mrcl, Mgl1 Retlna and Arg1 in Vehicle (EtOH) or 25-hydroxycholesterol (25HC)-treated BMMΦ in response to IL-4. mRNA expression over BK as mean ± SEM of n=4 biological replicates per group. f, Expression of the macrophage alternative activation markers of Mrcl, Arg1 and Mgl1 in WT and SREBP1c-KO BMMΦ in response to IL-4. mRNA expression over BK of n=3 biological replicates. g, mRNA expression over BK of RAW264.7 macrophages transfected with siRNA against SREBP1, SREBP2 or both in response to IL-4. Data is expressed as mean ± SEM of n=6 different experiments. Data has been analysed using a 2-way ANOVA followed by Sidak (a, b, d-f) or Tukey (g) post-hoc test.
Extended Data Fig. 4 | Macrophage SREBP1 activation is required for immune response to helminth infection. a, Neutrophil number presented as mean ± SEM in the lungs of naïve or 5- and 7-days post N. brasiliensis inoculation in WT and SCAP-KO mice. b, Eosinophil number presented as mean ± SEM in the lungs of naïve or 5- and 7-days post N. brasiliensis inoculation in WT and SCAP-KO mice. c, Percentage of alternatively activated alveolar macrophages in the lungs of naïve 5- and 7-days post N. brasiliensis inoculation in WT and SCAP-KO mice within the alveolar macrophage population. Alternative polarization was quantified by the expression of RELMα and CD206 by flow cytometry. d-f, Interstitial macrophages number (d), percentage of alternative activation (e) and number of alternatively activated interstitial macrophages (f) presented as mean ± SEM in the lungs of naïve or 5- and 7-days post N. brasiliensis inoculation in WT and SCAP-KO mice. g and h, Alveolar (g) and interstitial (h) neutrophil histological score in the lungs of naïve or 5- and 7-days post N. brasiliensis inoculation in WT and SCAP-KO mice. i, Correlation between the alveolar proteinaceous debris score and the number of alveolar macrophages 5 days post N. brasiliensis inoculation in WT and SCAP-KO mice. Pooled data as mean ± SEM n = 6-12 mice per group from 2 independent experiments. Data was analyzed using a two-way ANOVA followed by Sidak post-hoc test for comparison between genotypes at different days of post inoculation (a-h) or linear regression modelling (i).
Extended Data Fig. 5 | Fatty acid synthesis in response to IL-4 requires SREBP1 activation. a and b, Gene enrichment analysis of the pathways associated with de novo lipogenesis and SREBP activation from RNA sequencing comparing CTR and IL-4-treated BMM or of the interaction effect of IL-4 in WT and SCAP-KO BMM. Data from n = 6 biological replicates per group. c, Lipid synthesis rate in Lipopolysaccharide (LPS) or IL-4-treated BMM. The data represents the incorporation of radiolabelled 14C-acetate in the lipid fraction as mean ± SEM of n = 4 biological replicates per group. d, Proportion of newly synthesized palmitate per hour in control or IL-4 stimulated BMM. The data are presented as mean ± SEM of n = 4 biological replicates. e, Exogenous palmitate uptake rate of control or IL-4 stimulated BMM. The data are presented as mean ± SEM of n = 4 biological replicates. f, FAME composition in order of increasing chain length and desaturation of WT and SCAP-KO BMM in response to IL-4. Data is presented as mean ± SEM of n = 4 biological replicates. g, Total, essential and non-essential fatty acid content of WT and SCAP-KO BMM in response to IL-4. Data is presented as mean ± SEM of n = 4 biological replicates. h, Exogenous palmitate uptake rate of WT and SCAP-KO BMM in response to IL-4. The data are presented as mean ± SEM of n = 4 biological replicates. Statistical analysis of the RNAseq data is detailed in the methods section. Data has been analysed using a 2-way ANOVA followed by a Dunnett (c) or Sidak post-hoc test (f-h) or a two-tailed Student’s t-test (d and e).
Extended Data Fig. 6 | Fatty acid but not cholesterol synthesis is required for macrophage alternative activation. 

a. Lipid synthesis in BMMφ pre-treated with increasing doses of the FASN inhibitors C75 and cerulenin (Cer) 30 minutes prior 24h IL-4 stimulation. The data represents the incorporation of radiolabelled 14C-acetate in the lipid fraction as mean ± SEM of n = 7 biological replicates from 2 independent experiments. 

b. Expression of the macrophage alternative activation markers Mrc1, Mgl2, Arg1, Retlna and Il4i1 in Vehicle (DMSO) or C75 (10µM)-treated BMMφ in response to IL-4. mRNA expression over BK as mean ± SEM of n = 4 biological replicates per group. 

c. Alternative activation of BMMφ in response to IL-4 and pre-treated with increasing doses of Cerulenin (Cer). Alternative activation was assessed by flow cytometry using the co-expression of RELMα and CD301. The quantification of the number of M(IL-4) macrophages as mean ± SEM of n = 4 biological replicates is presented in d. 

d. Expression of the macrophage alternative activation markers Retlna and Mgl1 in Vehicle (DMSO) or cerulenin (2.5µg/mL)-treated BMMφ in response to IL-4. mRNA expression over BK as mean ± SEM of n = 4 biological replicates per group. 

e. Expression of the inflammatory cytokine Tnf and Il1b in Vehicle (DMSO) or C75 (10µM)-treated BMMφ in response to IL-4. mRNA expression over BK as mean ± SEM of n = 4 biological replicates per group. 

f. Expression of the SREBP2-target genes and macrophage activation markers Tnf, Retlna and Mgl2 in Vehicle (DMSO) or Simvastatin (10µM)-treated BMMφ in response to IL-4. mRNA expression over BK as mean ± SEM of n = 4 biological replicates per group. 

i. mRNA expression of the macrophage activation markers Retlna and Mgl2 in Vehicle (DMSO) or C75 (10µM)-treated BMMφ in response to IL-4 supplemented or not with HMG-CoA (1mM). mRNA expression over BK as mean ± SEM of n = 4 biological replicates per group. Data has been analysed using a 2-way ANOVA followed by a Dunnett (a and d) or Sidak post-hoc test (e-h) or one-way ANOVA followed by Tukey post-hoc test (i).
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Fatty acid or cholesterol supplementation does not rescue the alternative activation of SCAP-KO or C75-treated macrophages. mRNA expression of the SREBP1 target genes *Fasn* and *Scd2* in C75-treated (a) or SCAP-KO (c) macrophages and of the macrophages alternative activation markers in C75-treated (b) or SCAP-KO (d) macrophages in response to IL-4 and/or palmitic acid (PA, 10 or 50 µM), oleic acid (OA, 10 or 50 µM) or water-soluble cholesterol (50 µM). mRNA expression of BK of n = 4 (C75) or n = 3 (SCAP-KO) biological replicates. Data has been analysed using a 2-way ANOVA followed by a Tukey post-hoc test.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Sources of the ROS in M(IL-4) cells. a, Gene enrichment analysis of the pathways associated with redox homeostasis and response to oxidative stress in IL-4 treated BMMΦ. Data from n = 6 biological replicates per group. Data of n = 6 biological replicates from 2 independent experiments per group. b, ROS accumulation in Vehicle (EtOH) or 25-hydroxycholesterol (25HC)-treated BMMΦ in response to IL-4. ROS were quantified by the fluorescence ratio of CM-H₂DCFDA over DNA (Hoechst). Data as mean ± SEM of n = 7 biological replicates from 2 independent experiments. c, ROS accumulation in Vehicle (DMSO) or cerulenin (1µg/mL)-treated BMMΦ in response to IL-4. ROS were quantified by the fluorescence intensity of CM-H₂DCFDA by flow cytometry. Data as mean ± SEM of n = 4 biological replicates. d, Reactive oxygen species (ROS) levels in BMMΦ pre-treated with DPI (NADPH oxidase inhibitor), Allopurinol (xanthine oxidase inhibitor) or L-NAME (nitric oxide synthase inhibitor) prior 24h stimulation with IL-4 or LPS. ROS were quantified by the fluorescence ratio of CM-H₂DCFDA over DNA (Hoechst). Data are presented as mean ± SEM of n = 4 biological replicates. e, Mitochondrial ROS production in response to IL-4. Mitochondrial ROS levels were determined by the fluorescence ratio of MitoSox over DNA (Hoechst). Data are presented as mean ± SEM of n = 8 biological replicates from 2 independent experiments. f, Time-course of fatty acid oxidation in response to IL-4 (10ng/mL). Data of n = 4 biological replicates. g and h, Oxygen consumption rate (OCR) in WT and SCAP-KO BMMΦ (g) or in Vehicle (DMSO) or C75 (10µM)-treated BMMΦ (h) in control or IL-4 stimulated macrophages. OCR was monitored using an XF-96 Extracellular Flux Analyzer following the sequential treatments with oligomycin (oligo), FCCP and rotenone/antimycin (R/A). Data are presented as mean ± SEM of n = 8 (WT vs KO) and n = 8 from 2 independent experiments (DMSO vs C75) biological replicates per group. i, Fatty acid oxidation in IL-4 treated BMMΦ in response to the AMPK activator AICAR (100 or 500 µM). Data of n = 4 biological replicates. j, Lipid synthesis in BMMΦ treated with increasing doses of the AMPK activator AICAR in response to IL-4 (10ng/mL, 24h). Data of n = 4 biological replicates. k, Fatty acid oxidation assay of SCAP-KO macrophages in response to IL-4 (10ng/mL, 24h). Etomoxir (40 µM) was used as a negative control for FAO. Data of n = 4 biological replicates. l, Fatty acid oxidation assay in control or IL-4 stimulated macrophages treated or not with C75 (10µM) or Cerulenin (1µg/mL) for 24h. Data of n = 4 biological replicates. m, ROS levels in C75 (10 µM) and/or Etomoxir (ETO, 40 µM)-treated BMMΦ in response to IL-4. ROS were quantified by the fluorescence ratio of CM-H₂DCFDA over DNA (Hoechst). Data of n = 4 biological replicates. n and o, Mitochondrial ROS production in WT and SCAP-KO BMMΦ (n = 12 biological replicates from 3 independent experiments) (n) or in Vehicle (DMSO) or C75 (10µM)-treated (n = 12 biological replicates from 3 independent experiments) BMMΦ (o) in M(IL-4) macrophages. Mitochondrial ROS levels were determined by the fluorescence ratio of MitoSox over DNA (Hoechst). p, Reduced glutathione (GSH) levels in C75 (10µM)-treated BMMΦ in response to IL-4. Data presented as mean ± SEM of n = 4 biological replicates. Data has been analysed using a 2-way ANOVA followed by a Sidak (b, c, k and n-p) or Dunnett (d and j) or Tukey (g and h) post-hoc test or a two-tailed Student’s t-test (e) or a one-way ANOVA followed by Dunnett post-hoc test (f and i).
Extended Data Fig. 9 | ROS scavenging impairs macrophage alternative activation. 

**a**, ROS levels in N-acetyl cysteine (NAC, 10 mM)-treated BMMΦ in response to IL-4. ROS were quantified by the fluorescence ratio of CM-H$_2$DCFDA over DNA (Hoechst). Data as mean ± SEM of n = 8 biological replicates from 2 independent experiments. 

**b and c**, Alternative activation of NAC-treated BMMΦ in response to IL-4. Alternative activation was assessed by the expression of RELMα and CD206 by flow cytometry. The quantification of the number of M(IL-4) macrophages is presented as mean ± SEM in c. Data of n = 8 biological replicates from 2 independent experiments. 

**d**, mRNA expression over BK of Arg1, Mgl1 and Retnla in NAC-treated BMMΦ in response to IL-4. Data as mean ± SEM of n = 4 biological replicates. Data was analysed using a two-way ANOVA followed by Sidak post-hoc test.
Extended Data Fig. 10 | Schematic representation of the mechanism by which DNL is activated and sensed in alternatively activated macrophages.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a

- □ □ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

- □ □ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

- □ □ The statistical test(s) used AND whether they are one- or two-sided. Only common tests should be described solely by name; describe more complex techniques in the Methods section.

- □ □ A description of all covariates tested

- □ □ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

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- □ □ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.

- □ □ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

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- □ □ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

**Data collection**

- BD FACSDIVA Software BD Version 8.0.1 (flow cytometry), MassHunter Workstation Software (GC-MS), Biorad Image Lab v6.1.0 (western blot), QuantStudio Software v1.3 (RT-PCR).

**Data analysis**

- Howto v1.0 (flow cytometry), MassHunter Workstation Software Quantitative Analysis Agilent Technologies Inc Version 8.07.00 (GC-MS), Microsoft Excel, GraphPad Prism v9.1.0, Qiangen Ingenuity Pathway Analysis, Fiji (https://imagej.net/software/Fiji/).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
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All the data presented in this manuscript is available upon reasonable request to the corresponding authors. Transfer of materials requires materials transfer agreements. The RNA sequencing dataset is deposited in Gene Expression Omnibus (GEO) under accession number GSE179066.
Field-specific reporting

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
For power calculations, alpha was set at 0.05 and 1-beta at 0.8. For the N. Brasiliensis experiment, we performed a pilot experiment using 7 CS/128/GU WT mice per group to determine the SD of macrophage alternative activation and seek for an effect size of 1.5 fold-change as defined by the change in alternative activation observed in between WT and SCAP-KO in our in vitro experiments. For infected mice a sample size of n=10 was required. Based on historical BMDM experiments from the lab, we estimated that n=4 per group would be sufficient to detect a 1.5 fold change with a SD of 0.25. No power calculations were performed for the IL-4c experiment.

**Data exclusions**
Exclusion criteria were pre-established (ROUT test (Q=1%)). One mouse was removed from the N. Brasiliensis infection experiment as it was a significant outlier for RBC and worm counts and was not further analysed. No other data was excluded in the study.

**Replication**
The IL-4c experiment has been performed twice, once for RNA collection, once for protein collection. The N. Brasiliensis experiment was performed in two batches and the data of the 2 batches was pooled, as stated in the figure legends. In vitro experiment were performed using at least 3-4 biological replicates (each point represent a single animal) as stated in the figure legends. In some experiments, pooled data of different BMDM cultures are presented as detailed in the figure legends. All presented experiments have been performed at least twice, apart from the Srebo1c-KO BMDM and the HMG-CoA supplementation experiments that have been performed only once using respectively 3 and 4 mice per group.

**Randomization**
For the IL-4c experiment, littermates were randomly allocated to either the PBS or IL-4c group. For the N. Brasiliensis experiment, WT and SCAP-KO were allocated into the different days of infection groups ensuring even distribution of the genotypes and to maximize the use of littermate controls.

**Blinding**
For the N. Brasiliensis experiments, random numbers were assigned to the animals after allocation to the groups by another researcher and the samples were provided to the investigator for analyses without knowledge of the genotype. Blinding was revealed after analysis of all the different readouts.

For IL-4c and the in vitro bone-marrow derived macrophages and RAW264.7 experiments, investigators were not blinded as a single individual was responsible for the experimental set-up, data collection and data analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

**Materials & experimental systems**

| n/a | Involved in the study |
| --- | -----------------------|
| ☐ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology |
| ☑ | Animals and other organisms |
| ☐ | Human research participants |
| ☒ | Clinical data |

**Methods**

| n/a | Involved in the study |
| --- | -----------------------|
| ☑ | ChIP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

**Antibodies**

**Antibodies used**

A detailed list of the antibodies used for this study is presented in the Extended data Fig. 2.

**Validation**

All the antibodies were validated by trusted supplier for flow cytometry and western blot. RRIDs (https://scicrunch.org/resources) for all the used antibodies are presented in the Extended data Fig. 2.
Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s)          | RAW264.7 were purchased from ATCC |
|-----------------------------|-----------------------------------|
| Authentication              | The used cell line was not authenticated |
| Mycoplasma contamination     | Mycoplasma contamination was not tested |
| Commonly misidentified lines| Name any commonly misidentified cell lines used in the study and provide a rationale for their use. |

Animals and other organisms

Policy information about studies involving animals. ARRIVE guidelines recommended for reporting animal research

| Laboratory animals          | Details regarding mice strain are fully provided within the Extended data Table 1. Only male mice were used. For the N. Brasiliensis mice were 2-4 months old and age-matched. For BMDM experiments, animals were 2-4 months old and littermate controls were used. Animals were housed in a temperature-controlled room (22°C) with 55% relative humidity (breeding and BMDM experiments) or 45-65% relative humidity (N. Brasiliensis infection experiments) and with a 12-h light/dark cycle. Food and water were available ad libitum. |
|-----------------------------|----------------------------------------------------------------------------------|
| Wild animals                | This study did not involved wild animals. |
| Field-collected samples     | No samples were collected from the field. |
| Ethics oversight            | This research has been regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB) and Medical Research Council (MRC) ARE5 animal facility, under pathogen-free conditions and housed according to UK Home Office guidelines. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Lungs were minced with scissors and digested for 30 minutes at 4 °C in RPMI1640 with 2.5 mg/ml collagenase I and 0.25 mg/ml DNase I. The digest was sequentially passed and mashed through a 70 μm cell strainer and the strainer washed with FACS buffer. The cell suspension was filtered with a 50 μm cell strainer and red blood cells were lysed. Cells were counted using Countess automated cell counter (Invitrogen) and further process for multicore flow cytometry. Cells were kept on ice until use or analysis. PBS or IL-4c injected mice were killed by cervical dislocation, peritoneal exudate cells (PECs) were harvested by lavage of the peritoneal cavity with sterile PBS. Total peritoneal exudate cells were counted after red blood cell lysis (Biolegend) using Countess II automated cell counter (Invitrogen) and further process for magnetic-activated cell sorting (MACS). Cells were kept on ice until use or analysis.

Instrument

BD LSR-Fortessa equipped with 5 lasers (UV 355nm, violet 405nm, blue 488nm, yellow 561nm and red 640nm)

Software

Data was acquired using BD Diva software and analysed using FlowJo v10.

Cell population abundance

A minimum of 5,000 events of the relevant cell population was used. Purity was assessed as defined in the gating strategy section for the N. Brasiliensis experiment. For BMDMs, we considered BMDMs cells expressing high levels of CD11b and F4/80. In BMDM cultures, >90% of the cells expressed CD11b and F4/80.
Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.