Sterol regulatory element–binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity

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Newly activated CD8+ T cells reprogram their metabolism to meet the extraordinary biosynthetic demands of clonal expansion; however, the signals that mediate metabolic reprogramming remain poorly defined. Here we demonstrate an essential role for sterol regulatory element–binding proteins (SREBPs) in the acquisition of effector-cell metabolism. Without SREBP signaling, CD8+ T cells were unable to blast, which resulted in attenuated clonal expansion during viral infection. Mechanistic studies indicated that SREBPs were essential for meeting the heightened lipid requirements of membrane synthesis during blastogenesis. SREBPs were dispensable for homeostatic proliferation, which indicated a context-specific requirement for SREBPs in effector responses. Our studies provide insights into the molecular signals that underlie the metabolic reprogramming of CD8+ T cells during the transition from quiescence to activation.

A hallmark of adaptive immunity is the ability of responding T lymphocytes to undergo extensive clonal expansion to protect the host from invading pathogens. To fulfill the requirements of clonal expansion, activated T cells must acquire distinct metabolic programs that meet heightened metabolic demands. Quiescent, naive T cells rely on mitochondrial oxidative phosphorylation and catabolic metabolism to fulfill the minimal energetic and biosynthetic requirements associated with quiescence and long-term survival. After activation, T lymphocytes rapidly switch their metabolic program to rely predominantly on glycolysis in a process highly analogous to the Warburg effect, first described for tumor cells1. Additional changes in intermediary metabolism include heightened flux through the pentose-phosphate pathway and greater reliance on glutaminolysis. The ‘preferential’ use of glycolytic, pentose-phosphate and glutaminolytic pathways enables rapidly dividing T cells to satisfy the heightened metabolic demands of clonal expansion and cellular replication2,3. Accordingly, genetic and pharmacologic inhibition of glycolytic, glutaminolytic and lipid-biosynthetic pathways attenuates the survival, cell-cycle progression and effector differentiation of T cells4–9. Despite the importance of metabolic reprogramming for effector cell fate and function, the molecular events that link T cell antigen receptor (TCR) signaling with metabolic reprogramming remain enigmatic.

A key component in the metabolic reprogramming of effector T lymphocytes is rapid upregulation of lipid-biosynthetic pathways4,8. Early isotopomer-enrichment studies demonstrated that activation of lymphocytes results in a rapid increase in new biosynthesis of cholesterol and fatty acids4. Critically, the addition of specific cholesterol derivatives (such as oxysterols) to cultures results in diminished lipid biosynthesis and inhibits cell-cycle progression in G1 phase, which suggests a link between lipid metabolism and cell-cycle progression. Subsequent studies have shown that statins, which are pharmacologic inhibitors of HMG-CoA reductase (the rate-limiting enzyme in cholesterol biosynthesis), also inhibit mitogen-driven lymphocyte population expansion10. Moreover, it has been established that genetic and pharmacologic perturbations in sterol homeostasis, through the action of the liver X receptor transcriptional axis, also influence the cell-cycle progression, survival and effector function of T lymphocytes8,11. Thus, the regulation of intracellular lipid metabolism is critical for proper lymphocyte growth and function. However, the
molecular mechanisms that link mitogenic signaling to the lipido-anabolic program of activated lymphocytes remain poorly defined.

The sterol regulatory element–binding proteins SREBP1 and SREBP2 are basic helix-loop-helix–zip transcription factors with a well-defined role in the regulation of cellular lipid homeostasis. Mammals have two SREBP-encoding genes that express three SREBPs: SREBP1a and SREBP1c are produced via alternative transcriptional start sites in Srebf1, whereas Srebf2 encodes SREBP2. Canonical SREBP1c signaling ‘preferentially’ drives the expression of genes encoding molecules involved in the biosynthesis of fatty acids, whereas SREBP2 predominately transactivates genes encoding molecules involved in cholesterol biosynthesis, intracellular lipid movement and lipoprotein import. The SREBP1a isoform is able to transactivate genes that are targets of both SREBP1c and SREBP2. In addition to their function in regulating the expression of genes encoding molecules involved in lipid biosynthesis and transport, SREBPs also transactivate key genes encoding molecules involved in the oxidative pentose-phosphate pathway and generation of the coenzyme NADPH, which ensures the availability of sufficient reducing equivalents to meet anabolic demands.

The influence of SREBP signaling on the metabolism and function of T cells is not well understood. Here we use genetic and pharmacological models to demonstrate that SREBPs were essential for CD8+ T cells to undergo metabolic reprogramming in response to mitogenic signaling. Loss of SREBP function in CD8+ T cells rendered them unable to efficiently blast, which resulted in diminished proliferative capacity in vitro and attenuated clonal expansion during viral infection. SREBP activity was required for the upregulation of glycolytic and oxidative metabolism after activation and for the maintenance of lipid content sufficient to permit rapid membrane biogenesis and cellular growth. We traced the defect in growth and proliferation to a deficiency in cellular cholesterol during blastogenesis, which was restored by cholesterol supplementation. However, SREBPs were dispensable for homeostatic proliferation, which indicated a specific requirement for SREBP signaling to meet the substantial anabolic demands that support effector responses. Our study provides mechanistic insights into the metabolic networks that ensure the growth of effector CD8+ T cells after activation and highlights the importance of SREBPs in regulating a cholesterol metabolic checkpoint during blastogenesis.

RESULTS
The TCR induces a lipid-biosynthesis program
T cells rapidly upregulate a program for the biosynthesis of fatty acids and cholesterol after stimulation with polyclonal mitogens. To understand the relationship between mitogenic stimulation and induction of the lipogenic program, we activated purified mouse T cells for 6 h with plate-bound antibody to CD3 (anti-CD3) alone or in combination with anti-CD28. Quantitative RT-PCR analysis showed that anti-CD3 alone was sufficient for the induction of genes encoding molecules involved in cholesterol biosynthesis (Hmgcr, Hmgcs and Sgce) and the biosynthesis of fatty acids (Acaca and Fasn), and the addition of anti-CD28 did not provide additional induction of the program (Fig. 1a). Activation of Pmel CD8+ T cells (which have transgenic expression of a TCR specific for the melanocyte-differentiation antigen gp100) with increasing doses of agonist gp100 peptide confirmed the requirement for TCR signaling and showed that induction of the lipogenic program occurred at sub-mitogenic doses of antigen (Supplementary Fig. 1a). Activation of T cells with the phorbol ester PMA induced the lipogenic program to amounts similar to those achieved by crosslinking of the TCR (Fig. 1a).

Induction was inhibited by pretreatment of cells with Gö6983, an inhibitor of protein kinase C (Supplementary Fig. 1b), which suggested a dependence on protein kinase C. In contrast, culture of cells with the calcium ionophore ionomycin alone did not induce genes encoding molecules involved in lipogenesis (Fig. 1a). Together these data indicated that signaling via the TCR and activation of protein kinase C were necessary and sufficient for induction of the lipogenic program in mitogen-stimulated T cells.

Published studies have indicated that loss of Tsc1, a negative regulator of signaling via the kinase mTOR, results in the upregulation of genes encoding molecules involved in sterol biosynthesis and propanoate metabolism in naive CD4+ T cells. Thus, we sought to determine if induction of lipid-biosynthesis programs by mitogens was dependent on mTOR signaling. To address this, we pretreated purified splenic T cells for 30 min with 100 nM rapamycin (an inhibitor of mTOR) or vehicle and then stimulated the cells for up to 6 h with anti-CD3 and anti-CD28. As expected, activation of T cells robustly induced genes encoding molecules involved in the biosynthesis of fatty acids and cholesterol, whereas pretreatment of cultures for 30 min with rapamycin completely inhibited induction of the lipogenic program (Fig. 1b). Immuno blot analysis of whole-cell and nuclear extracts showed that rapamycin prevented the processing of full-length SREBP, which resulted in no accumulation of mature SREBP in the nucleus (Supplementary Fig. 1c). Inactivation of the phosphatidylinositol-3-OH kinase (PI(3)K) pathway by treatment with LY294002 similarly repressed genes encoding molecules involved in lipogenesis (Fig. 1b). These data indicated that signaling through the PI(3)K-mTOR pathway was necessary for the induction of genes encoding molecules involved in the lipid-anabolic program of activated lymphocytes.

SREBPs mediate the lipid-anabolic program of T cells
To directly address the function of SREBPs in induction of the lipogenic program by mitogenic signals, we retrovirally transduced purified splenic and lymph node T cells with a truncated, constitutively active form of SREBP1a (ΔSREBP1a) or SREBP2 (ΔSREBP2; Supplementary Fig. 1d,e). We collected mRNA 24 h after retroviral infection and assessed expression of genes encoding molecules involved in lipid biosynthesis by RT-PCR. Cells infected to express ΔSREBP1a or ΔSREBP2 had much higher expression of genes encoding molecules involved in the biosynthesis of fatty acids and cholesterol than did control cells infected with retrovirus containing empty vector (Fig. 1c), which indicated that the lipogenic program of T cells was responsive to SREBP signaling.

In complementary experiments, we sought to determine if SREBPs were required for induction of the lipogenic program by mitogenic signaling. To directly address this, we transiently transfected purified mouse spleen and lymph node T cells with small interfering RNA (siRNA) targeting SREBP1 and/or SREBP2 and then stimulated the cells with PMA and ionomycin. After 5 h of stimulation, we collected mRNA and assessed expression of genes encoding molecules involved in lipid biosynthesis by RT-PCR. Cells infected to express ΔSREBP1a or ΔSREBP2 had much higher expression of genes encoding molecules involved in the biosynthesis of fatty acids and cholesterol than did control cells infected with retrovirus containing empty vector (Fig. 1d), which indicated that the lipogenic program of T cells was responsive to SREBP signaling.
Figure 1 The lipid-biosynthesis program of activated T cells is SREBP dependent and sensitive to the PI(3)K-mTOR pathway. (a) Real-time PCR analysis of genes encoding molecules involved in lipogenesis in mouse spleen and lymph node T cells activated for 6 h with plate-bound anti-CD3 alone (CD3) or with anti-CD28 (CD3 + CD28), or with PMA or ionomycin (Iono) alone or together (PMA + Iono); results are presented relative to those of quiescent T cells (Ex vivo), set as 1. * P < 0.05 and ** P < 0.01 (two-tailed unpaired Student’s t-test). (b) Real-time PCR analysis of gene expression in T cells pretreated for 30 min with vehicle, the PI(3)K inhibitor LY294002 (LY294; 10 µM) or the mTOR inhibitor rapamycin (Rap; 100 nM), then activated for 6 h with anti-CD3 and anti-CD28; results are presented relative to those of quiescent T cells (Ex vivo), set as 1. * P < 0.05, LY294002 versus vehicle, ** P < 0.05, rapamycin versus vehicle, ** P < 0.01, LY294002 versus vehicle, and †† P < 0.01, rapamycin versus vehicle (two-tailed unpaired Student’s t-test). (c) Real-time PCR analysis of genes encoding molecules involved in lipogenesis in activated T cells transduced for 24 h with active SREBP (ΔSREBP1a or ΔSREBP2) or empty vector (EV); results are presented relative to those of uninfected T cells (UI), set as 1. * P < 0.05, empty vector versus ΔSREBP1a, † P < 0.05, empty vector versus ΔSREBP2, ** P < 0.01, empty vector versus ΔSREBP1a, and †† P < 0.01, empty vector versus ΔSREBP2 (two-tailed unpaired Student’s t-test). (d) Real-time PCR analysis of gene expression in T cells transduced with siRNA targeting SREBP1 alone (siSREBP1) or siSREBP2 alone (siSREBP2), or both together (siSREBP1+2) or nontargeting control siRNA (siCtrl), then activated for 5 h with PMA and ionomycin; results are presented relative to those of quiescent T cells (Ex vivo), set as 1. * P < 0.05, siCtrl versus siSREBP1, † P < 0.05, siCtrl versus siSREBP2, †† P < 0.01, siCtrl versus siSREBP1+2, ††† P < 0.001, siCtrl versus siSREBP1, and ††† P < 0.001, siCtrl versus siSREBP2 (two-tailed unpaired Student’s t-test). (e) ChIP analysis of the abundance of SREBP1 and SREBP2 at the promoters of various genes (vertical axes) in splenocytes immediately after isolation (Ex vivo) or splenocytes pretreated for 30 min with vehicle, rapamycin (100 nM) or 25-hydroxycholesterol (25-HC; 10 µM) and then activated for 4 h with PMA; results are normalized to input and are presented relative to those obtained with immunoglobulin G (IgG). Rplp0, control gene not targeted by SREBPs. Inset, immunoblot analysis of phosphorylated (p-) and total S6 in whole-cell lysates of cells treated as in graphs (to confirm rapamycin function). * P < 0.05, vehicle versus rapamycin, † P < 0.05, vehicle versus 25-HC, ** P < 0.01, vehicle versus rapamycin, †† P < 0.001, vehicle versus 25-HC, *** P < 0.001, vehicle versus rapamycin, and ††† P < 0.001, vehicle versus 25-HC (two-tailed unpaired Student’s t-test). Data are representative of at least three experiments (mean and s.d. of triplicates).

We were able to achieve only partial knockdown of SREBP1 with siRNA targeting SREBP1 (Supplementary Fig. 1f) and, correspondingly, we observed a small effect on genes encoding molecules involved in the synthesis of cholesterol and fatty acids (Fig. 1d). The observation that overexpression of ΔSREBP1α or ΔSREBP2 resulted in upregulation of genes encoding molecules involved in the biosynthesis of fatty acids and cholesterol in activated T cells led us to hypothesize that SREBP1 and SREBP2 might act together, or share occupancy, at the promoters of genes encoding molecules involved in lipogenesis. Thus, we analyzed lysates of quiescent and activated T cells by chromatin immunoprecipitation (ChIP) of SREBP1 and SREBP2. In quiescent cells, SREBP2 was readily detectable at the promoters of Hmgcr and Hmgcs, which are canonical target genes of SREBP2 (Fig. 1e). Activation of T cells for 4 h with PMA resulted in tenfold or greater enrichment in SREBP2 at the promoters of Hmgcr and Hmgcs (Fig. 1e). Enrichment of SREBP1 was also detectable at the promoters of Hmgcr and Hmgcs. Likewise, we found both SREBP1 and SREBP2 at the promoters of genes encoding molecules involved in the synthesis of fatty acids (Fig. 1e). These data suggested that both SREBP1 and SREBP2 may have acted together in driving the lipogenic program of activated T cells. Pretreatment of cultures with 10 µM 25-hydroxycholesterol, a well-described sterol inhibitor of SREBP processing, resulted in much less enrichment of SREBP1 and SREBP2 at the promoters of various genes encoding molecules involved in lipogenesis (Fig. 1e) and, consequently, induction of the lipid-synthesis program (Supplementary Fig. 1g). Finally, pretreatment of cultures with 100 nM rapamycin also diminished the enrichment for SREBP1 and SREBP2 to background (Fig. 1e), which further indicated that mTOR was needed to link TCR signaling with induction of the lipid-synthesis program via SREBP.

Deletion of the chaperone SCAP inhibits SREBP activity in T cells Published studies of SREBPs indicate that SREBP1 and SREBP2 can compensate for each other when one protein is deleted15,16. Moreover, our overexpression and ChIP studies suggested that both SREBP1 and SREBP2 could potentially influence the lipid-biosynthesis program in mitogen-stimulated T cells. Thus, we reasoned that loss of both SREBP1 and SREBP2 would be required for full ablation of the lipogenic program of activated T cells. To achieve this, we crossed mice with loxP-flanked alleles encoding the SREBP chaperone protein SCAP (‘SREBP cleavage-activating protein’; Scaplox/lox mice) with mice expressing Cre recombinase under control of the Cd4 gene (Cd4-Cre mice).
to achieve T cell–specific deletion of Scap in the Scapfl/flCd4-Cre progeny (called ‘Scapfl/fl’ here). SCAP is associated with SREBPs in the endoplasmic reticulum (ER) membrane and regulates the processing and subsequent transcriptional activity of both SREBP1 and SREBP2 (ref. 17). We found no difference between 5- to 6-week-old Scapfl/fl mice and their Scapfl/+Cd4-Cre littersmates (called ‘wild-type mice’ here) in the cellularity of thymus, spleen and lymph node (Fig. 2a). Phenotypic analysis of thymic and peripheral T cell subsets demonstrated no demonstrable difference between Scapfl/fl and wild-type mice in the frequency of CD4+ or CD8+ T cells (Fig. 2b). We also did not find a difference between these mice in the expression pattern of the activation markers CD25 and CD44 (Supplementary Fig. 2a) or in the total number of CD4+CD25+ and CD8+CD44+ cells (Supplementary Fig. 2b). Finally, we did pulse-chase experiments with the thymidine analog BrdU to monitor the homeostatic maintenance of peripheral T cells and found no substantial difference between these mice in labeling or decay over a 42-day period (Supplementary Fig. 2c).

Gene-expression studies confirmed nearly complete deletion of Scap in quiescent peripheral Scapfl/fl T cells, and we observed a modest change in the expression of genes encoding molecules involved in lipogenesis (Fig. 2c). ChIP studies demonstrated that T cell–specific deletion of Scap resulted in a slightly lower abundance of detectable SREBP protein at the promoters of target genes in quiescent cells (Fig. 2d). As expected, wild-type cells rapidly upregulated the lipid-biosynthesis program after activation (Fig. 2c), which led to more SREBP1 and SREBP2 at the promoters of target genes (Fig. 2d). In contrast, the induction of genes encoding molecules involved in lipogenesis was considerably attenuated in Scapfl/fl CD8+ T cells (Fig. 2c). Correspondingly, we observed a significantly lower abundance of SREBP1 and SREBP2 at promoters of genes encoding molecules involved in lipid biosynthesis (Fig. 2d). Together these data indicated that the SREBP-dependent lipid-biosynthesis program was largely dispensable for quiescent peripheral T cells; however, genetic deletion of Scap attenuated upregulation of the SREBP transcriptome of mitogen-stimulated T cells.

SREBPs influence CD8+ T cell growth and proliferation

To determine if loss of SREBP activity would influence T cell blastogenesis and proliferation in vitro, we labeled purified wild-type and Scapfl/fl CD8+ T cells with the cytosolic dye CFSE, then stimulated the cells for 72 h with anti-CD3 with or without anti-CD28 or IL-2. Wild-type T cells enlarged by 24 h (Fig. 3a and Supplementary Fig. 3a) and diluted CFSE by 72 h after activation with mitogens (Fig. 3b). In contrast, Scapfl/fl CD8+ T cells did not undergo substantial enlargement (Fig. 3a and Supplementary Fig. 3a) and proliferated only modestly in response to anti-CD3 (Fig. 3b). The addition of exogenous IL-2 and/or anti-CD28, or PMA and ionomycin, to cultures resulted in only slightly greater proliferation of Scapfl/fl T cells (Fig. 3b). Cell-cycle analysis indicated that activated Scapfl/fl CD8+ T cells remained in the G0-G1 phases of the cell cycle (Fig. 3c). The addition of anti-CD28 and/or IL-2 to cultures resulted in a slightly greater proportion of cells in the S and G2-M phases (Fig. 3c). These data indicated that SREBP signaling was essential for mitogen-driven blastogenesis, proliferation and survival of CD8+ T cells.

Our cell-cycle data indicated that Scap-deficient T cells remained in the G0-G1 phases. To better define whether Scap-deficient T cells were able to move out of the G0 phase into the G1 phase of the cell cycle, we assessed expression of G1 phase–associated cyclins and restriction-point proteins. Immunoblot analysis of wild-type and Scapfl/fl CD8+ T cells activated for 18 h indicated that Scapfl/fl T cells upregulated cyclin D2 and cyclin D3 (Fig. 3d). We also observed less phosphorylation of the cell-cycle-checkpoint protein Rb and maintenance of the cell-cycle inhibitor p27kip1 (Fig. 3d), which suggested that Scapfl/fl cells were moving into the G1 phase of the cell cycle but were not entering S phase. We also determined if the loss of SREBP activity resulted in apoptosis. However, kinetic studies indicated no difference between wild-type and Scapfl/fl cells in the frequency of cells positive for cleaved caspase-3 up to 36 h after activation (Fig. 3e). Similarly, we observed no difference between wild-type and Scapfl/fl cells in...
Loss of SREBPs affects the lipid homeostasis of T cells

Next we sought to determine if genetic ablation of SREBP activity grossly perturbed lipid homeostasis in T cells. To address this, we purified splenic T cells from 5- to 8-week-old Scapfl/fl mice and their wild-type littermates. We measured the total content of cholesterol and fatty acids (myristate, palmitate and stearate) by gas chromatography-mass spectrometry immediately after isolation. T cells from Scapfl/fl mice had approximately 50% less cellular cholesterol than did T cells from their wild-type littermates (Fig. 4a). Quiescent Scapfl/fl T cells also had slightly less palmitate than did their wild-type counterparts (Fig. 4b); however, we observed no significant difference between resting wild-type and Scapfl/fl T cells in myristate or stearate. Activation of wild-type T cells for 24 h with anti-CD3 and anti-2B resulted in more cholesterol, myristate, palmitate and stearate on a per-cell basis than that in freshly isolated cells (Fig. 4b). In contrast, Scapfl/fl T cells did not increase their abundance of cholesterol, myristate, palmitate or stearate in response to activation (Fig. 4a,b). Together these data indicated that Scap-deficient T cells moved efficiently into the G1 phase of the cell cycle but were unable to exit this phase before undergoing caspase-independent death.

SREBPs regulate a gene program required for T cell growth

To better understand how SREBP was influencing CD8+ T cell growth and survival, we did global gene-expression studies of quiescent CD8+ T cells and CD8+ T cells stimulated for 6 h with anti-CD3 and anti-CD28. Many genes were either upregulated or downregulated in wild-type CD8+ T cells after stimulation with anti-CD3 and anti-CD28 (Fig. 5). Notably, we observed a very similar pattern of gene expression in activated Scapfl/fl cells, which indicated that loss of SREBP activity did not globally perturb T cell activation. Pathway analysis of genes with a significant difference in expression in quiescent wild-type CD8+ T cells relative to their expression in Scapfl/fl CD8+ T cells identified a small set of genes encoding molecules involved in cellular survival that were downregulated in the absence of SREBP activity (P < 0.001; Supplementary Table 1).

Next we did pathway analysis of genes with significantly less induction in Scapfl/fl CD8+ T cells than in wild-type cells after activation with anti-CD3 and anti-CD28 through the use of the DAVID bioinformatics database (Database for Annotation, Visualization and Integrated Discovery)20,21. This analysis showed that activated Scapfl/fl cells in phosphorylation of Akt and its downstream target S6 in response to activation (Fig. 4d). Moreover, activation with PMA and ionomycin, which obviates the requirement for signaling via the TCR and costimulation, did not provide substantial restoration of the cell-cycle progression, viability and proliferative ability of Scapfl/fl T cells (Fig. 3b,c). These data suggested that it was unlikely that the inability of Scap-deficient T cells to grow was a function of changes in signaling via the TCR and coreceptors.
Figure 4 Loss of SREBP signaling affects lipid homeostasis but does not perturb proximal TCR signaling. (a,b) Abundance of cholesterol (a) and long-chain fatty acids (b) in wild-type and Scap<sup>fl/fl</sup> T cells, assayed by gas chromatography–mass spectrometry in cells immediately after isolation (0) or after activation for 24 h with anti-CD3 and anti-CD28 (24); results are normalized to cell numbers and are presented relative to those of unactivated wild-type cells. (c) Immunoblot analysis of total and phosphorylated (p-) proteins in whole-cell lysates of wild-type and Scap<sup>fl/fl</sup> lymphocytes left unstimulated (0 h) or stimulated for 5, 30 or 60 min (above lanes) with soluble anti-CD3e (1 μg/ml). MAPK, mitogen-activated protein kinase. (d) Immunoblot analysis of the Akt pathway in wild-type and Scap<sup>fl/fl</sup> CD8<sup>+</sup> T cells assessed immediately after isolation or after activation for 6 h with anti-CD3 and anti-CD28. p-Akt (S473), Akt phosphorylated at Ser473. NS, not significant; *P < 0.05, **P < 0.01 and ***P < 0.001 (two-tailed unpaired Student’s t-test). Data are representative of one experiment with three replicates per group (a,b, mean and s.d. of triplicates) or three experiments (c,d).

Metabolic reprogramming requires SREBP
To further investigate the metabolic state of Scap-deficient T cells, we analyzed the cellular bioenergetics of quiescent wild-type and Scap<sup>fl/fl</sup> T cells. We observed no difference between freshly isolated wild-type and Scap<sup>fl/fl</sup> CD8<sup>+</sup> T cells in their basal extracellular acidification rate (ECAR) or oxygen-consumption rate (OCR; Fig. 6a). Stimulation of wild-type CD8<sup>+</sup> T cells for 24 h with anti-CD3 and anti-CD28 resulted in a tenfold greater ECAR and a fourfold greater OCR. In contrast, Scap<sup>fl/fl</sup> CD8<sup>+</sup> T cells stimulated similarly had only a slightly greater OCR and ECAR and were unable to achieve the rates in wild-type cells.

Next we directly assessed mitochondrial function (ATP production and spare respiratory capacity) in Scap-deficient CD8<sup>+</sup> T cells by monitoring the OCR in response to sequential treatment with the ATPase inhibitor oligomycin, the uncoupling agent FCCP and the electron-transport-chain inhibitors rotenone and myxothiazol (Fig. 6b). We observed no difference between freshly isolated wild-type and Scap<sup>fl/fl</sup> CD8<sup>+</sup> T cells in basal respiration, ATP production or spare respiratory capacity in response to treatment with oligomycin, FCCP and rotenone–myxothiazol (Fig. 6c). Activation of wild-type CD8<sup>+</sup> T cells for 24 h with anti-CD3 and anti-CD28 resulted in higher basal OCR than that in quiescent CD8<sup>+</sup> T cells (Fig. 6a,c). Treatment of activated wild-type CD8<sup>+</sup> T cells with oligomycin, FCCP and rotenone–myxothiazol also demonstrated greater mitochondrial ATP-production capacity and spare respiratory capacity in those cells than in their quiescent counterparts (Fig. 6c), consistent with enhanced mitochondrial function in ‘blasting’ CD8<sup>+</sup> T cells.

Figure 5 SREBP activity influences a transcriptional program related to lipid and RNA metabolism. Heat map of gene expression in purified quiescent wild-type and Scap<sup>fl/fl</sup> CD8<sup>+</sup> T cells (0) or cells stimulated for 6 h with anti-CD3 and anti-CD28 (6). Left margin, for genes with a change in expression with a P value of <0.001, less (Less (act)) or greater (More (act)) induction in activated Scap<sup>fl/fl</sup> CD8<sup>+</sup> T cells than in activated wild-type CD8<sup>+</sup> T cells, or less induction in quiescent Scap<sup>fl/fl</sup> CD8<sup>+</sup> T cells than in quiescent wild-type CD8<sup>+</sup> T cells (Less (q)). Data are representative of one experiment with three replicates per group.
in those pathways. However, immunoblot analysis indicated normal upregulation of c-Myc expression and more stabilization of HIF-1α in activated *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells than in their wild-type counterparts (Supplementary Fig. 4b). Thus, the inability of *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells to acquire a glycolytic program during activation was probably not a direct function of altered signaling via c-Myc or HIF-1α.

A logical prediction of our metabolic studies was that blasting Scap-deficient T cells would have altered bioenergetics. To directly address this, we measured intracellular ATP concentrations in quiescent CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells activated with anti-CD3 and anti-CD28. We observed no difference between wild-type and *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells in ATP concentration immediately after isolation (Fig. 6g). Activation of wild-type CD8<sup>+</sup> T cells resulted in a slightly greater abundance of ATP by 6 h and fourfold more by 24 h (Fig. 6g). In contrast, *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells did not have a greater cellular ATP abundance at 6 h and achieved only twofold more ATP at 24 h after stimulation than that in their unactivated counterparts (Fig. 6g). We considered the possibility that perturbations in the concentration of ATP would activate the AMP-activated protein kinase (AMPK) pathway in Scap-deficient CD8<sup>+</sup> T cells. Consistent with published studies, activation of wild-type T cells resulted in phosphorylation of AMPK and the AMPK target acetyl-CoA carboxylase (Supplementary Fig. 4c). We noted no difference between similarly activated *Scap*<sup>fl/fl</sup> T cells and wild-type CD8<sup>+</sup> T cells in their phosphorylation of AMPK and acetyl-CoA carboxylase (Supplementary Fig. 4c), which suggested that the difference between wild-type and *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells in glycolysis and cellular growth was not a function of exaggerated AMPK signaling. Together these data supported the idea that SREBP signaling influenced cellular bioenergetics during blastogenesis independently of the c-Myc, HIF-1α and AMPK pathways.

### Cholesterol restores growth of Scap-deficient T cell

Our gene-expression data indicated that loss of *Scap* influenced mainly lipid homeostasis, in particular flux through the mevalonate pathway. In addition to cholesterol synthesis, the mevalonate pathway is responsible for the generation of nonsteroidal lipid modification of proteins, such as prenylation of the GTPase Ras<sup>2,4</sup>. Thus, we sought to determine if the addition of excess mevalonic acid to cultures would increase flux into isoprenoid synthesis and restore the growth of Scap-deficient T cells. However, the addition of mevalonic acid (50–100 μM) did not affect survival or blastogenesis (Supplementary Fig. 5a), which suggested that a defect in the prenylation of proteins could not mechanistically explain the phenotype of *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells.

Those data led us instead to posit that cholesterol was limiting in *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells. To directly assess our hypothesis, we activated wild-type and *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells with anti-CD3 and anti-CD28 in complete medium supplemented with 5 μg/ml of cholesterol conjugated to methyl-β-cyclodextrin. Analysis of cultures up to 48 h showed that the addition of cholesterol to wild-type CD8<sup>+</sup> T cells afforded a modest improvement in cellular survival, with no influence on cell size.

### Table 2 Predicted transcription factors

| Transcription factor | P       | FDR     |
|----------------------|---------|---------|
| CREBBP               | 2.47 × 10<sup>-9</sup> | 4.55 × 10<sup>-3</sup> |
| SREBF2               | 2.48 × 10<sup>-9</sup> | 6.82 × 10<sup>-3</sup> |
| SREBF1               | 2.65 × 10<sup>-8</sup> | 9.09 × 10<sup>-3</sup> |
| NFYA                 | 3.86 × 10<sup>-5</sup> | 1.14 × 10<sup>-2</sup> |
| YY1                  | 6.69 × 10<sup>-4</sup> | 1.36 × 10<sup>-2</sup> |

Predicted transcription factors negatively regulated in Scap-deficient T cells for the list of genes induced less in *Scap*<sup>fl/fl</sup> CD8<sup>+</sup> T cells activated for 6 h with anti-CD3 and anti-CD28 than in their wild-type counterparts. P values, Fisher’s test; FDR, false-discovery rate. Data are representative of one experiment.
SREBPs intrinsically regulate virus-specific immunity

To determine if SREBP signaling was also required for efficient T cell clonal expansion in vivo, we challenged wild-type and Scapfl/fl mice with the Armstrong strain of lymphocytic choriomeningitis virus (LCMV). Infection of wild type mice with LCMV Armstrong generates a robust, virus-specific T cell response that leads to rapid control of viral infection within 10–12 d (ref. 26). On day 8 after infection, we counted splenocytes and stained them with LCMV-specific tetramers of H-2D\(^b\) and amino acids 33–41 of LCMV glycoprotein (gp33) to assess the abundance of virus-specific CD8\(^+\) T cells. Scapfl/fl mice had a much lower frequency and total number of CD8\(^+\) T cells specific for H-2D\(^b\)-gp33 than did wild-type mice (Fig. 8a–c). We obtained similar results for CD8\(^+\) T cells specific for tetramers of H-2D\(^b\) and amino acids 396–404 of LCMV nucleoprotein (Fig. 8e), which indicated a globally diminished antiviral T cell response in Scapfl/fl mice. Intracellular staining of interferon-\(\gamma\) and tumor-necrosis factor after challenge with gp33 peptide ex vivo confirmed a lower frequency of responding CD8\(^+\) T cells in Scapfl/fl mice than in wild-type mice (Fig. 8d,e). The LCMV-specific CD8\(^+\) T cells generated in Scapfl/fl mice had lower expression of interferon-\(\gamma\) and tumor-necrosis factor than did their wild-type counterparts (Fig. 8d), which suggested a potential functional defect in the absence of SREBP activity.

Finally, our data indicated an essential requirement for SREBPs in mitogen-driven proliferation in vitro and clonal expansion of viral-specific CD8\(^+\) effector cells in vivo. However, we did not observe a significant perturbation in the frequency or absolute number of peripheral T cells from naive Scapfl/fl mice (Fig. 2). Those data led us to posit that SREBP activity might not be required under homeostatic conditions. To initially address this, we examined the homeostatic proliferation of purified, CFSE-labeled CD8\(^+\) T cells adoptively transferred into Thy-1.1\(^+\) lymphopenic host mice (deficient in recombination-activating gene 2 (Rag2\(^{−/−}\))). On day 6 after adoptive transfer, we assessed the CFSE dilution of donor Thy-1.2\(^+\) wild-type or Scapfl/fl T cells from the lymph nodes and spleen of the recipient mice. Consistent with our hypothesis, we observed no difference in the proliferative patterns of wild-type and Scapfl/fl mice T cells (Fig. 8f). We also observed similar proliferative results in a sublethal-irradiation model.
Figure 7 The addition of cholesterol restores the growth and proliferation of Scap-deficient CD8+ T cells. (a) Viability of wild-type and Scapfl/fl CD8+ T cells activated for 48 h with anti-CD3 and anti-CD28 alone (Control) or in the presence of cholesterol (5 μg/ml) conjugated to methyl-β-cyclodextrin (Cholesterol) in medium supplemented with 10% FBS. Numbers adjacent to outlined areas indicate percent DAPI-negative (live) cells. (b) Flow cytometry of DNA content from wild-type and Scapfl/fl CD8+ T cells treated as in a. (c) Flow cytometry of DNA content from wild-type and Scapfl/fl CD8+ T cells treated as in a, assessed by staining with propidium iodide. Numbers above lines in plots indicate percent cells in the S and G2-M phases. (d) CFSE dilution by DAPI-negative (live) wild-type and Scapfl/fl CD8+ T cells treated as in a. (e) Staining of the ER (with ER-Tracker) in unactivated wild-type and Scapfl/fl CD8+ T cells (0 h) or cells activated for 6, 16 or 18 h with anti-CD3 and anti-CD28. Neg ctrl, unstained cells (negative control). (f) Staining of the ER (as in e) in wild-type and Scapfl/fl CD8+ T cells treated as in a. Data are representative of two experiments.

of homeostatic proliferation27 (Fig. 8f). By RT-PCR analysis, we found that homeostatically proliferating cells did not upregulate the SREBP-driven expression of genes encoding molecules involved in cholesterol synthesis, although we did observe very modest upregulation of Fasn (Fig. 8g). Thus, we posited that a chief difference between T cells undergoing homeostatic proliferation and those undergoing conventional antigen-driven proliferation might be cellular growth. Indeed, analysis of cell size indicated that homeostatically proliferating...

Figure 8 Loss of SREBP activity impairs the clonal expansion of antigen-specific effector CD8+ T cells but does not perturb homeostatic proliferation. (a) Absolute number of splenocytes and CD8+ T cells from spleens collected on day 8 after infection of wild-type and Scapfl/fl mice with LCMV Armstrong. (b) Flow cytometry of T cells in spleens from mice treated as in a. Numbers in quadrants indicate percent CD8+ T cells positive (top right) or negative (bottom right) for the H-2Db-gp33 tetramer (gp33-tet). (c) Absolute number of H-2Db-gp33 tetramer–specific (gp33-tet+) CD8+ T cells (left) and CD8+ T cells specific for a tetramer of H-2Db and amino acids 396–404 of LCMV nucleoprotein (NP396-tet; right) in spleens from mice treated as in a. (d,e) Frequency (d) and absolute number (e) of interferon (IFN-)γ- or tumor-necrosis factor (TNF)-producing wild-type and Scapfl/fl CD8+ T cells collected on day 8 after infection as in a and stimulated for 5 h in vitro with gp33 peptide. Numbers in far corners of quadrants indicate percent cells in each; numbers below in top right quadrants indicate mean fluorescence intensity (MFI) of cytokine-producing CD8+ T cells. (f) CFSE dilution of adoptively transferred wild-type or Scapfl/fl T cells from the spleen and lymph nodes of lymphopenic Thy-1.1+ Rag2−/− host mice (top) or their irradiated wild-type littermate hosts (bottom), on day 6 after transfer; CFSE-labeled wild-type or Scapfl/fl CD8+ T cells were gated on Thy-1.2+CD8+ T cells (top) or CD3+CD4+ or CD3+CD8+ cells (bottom). (g) Real-time PCR analysis of genes encoding molecules involved in lipogenesis in polyclonal Thy-1.1+ donor T cells sorted from spleens and lymph nodes of lymphopenic Thy-1.2+ Rag2−/− recipient mice on day 6 after adoptive transfer of Thy-1.1+ T cells; results are presented relative to those at day 0, set as 1. (h) Forward scatter of OT-I cells from lymphopenic Rag2−/− recipient mice given adoptive transfer of OT-I cells and then left untreated (UT) or immunized subcutaneously with ovalbumin in incomplete Freund’s adjuvant (Imm); cells obtained from the spleens and lymph nodes of the recipient mice 24 h later were stained and analyzed by flow cytometry (n = 3 mice per group). Pre, cells obtained from donor mice before transfer. *P < 0.05 and **P < 0.01 (two-tailed paired Student’s t-test). Data are representative of three (a-e) or two (f-h) experiments (error bars (a,c,e,g), s.d.).
CD8+ T cells from OT-I mice (which have transgenic expression of an ovalbumin-specific TCR) remained small despite undergoing significant proliferation in lymphopenic hosts. In contrast, OT-I CD8+ T cells adoptively transferred into lymphopenic hosts underwent conventional blastogenesis after immunization of the host with ovalbumin (Fig. 8h). Together these data demonstrated a context-specific requirement for SREBP signaling during conventional antigen-driven responses to support cellular enlargement and effector function.

**DISCUSSION**

The molecular events that guide the metabolic reprogramming of T cells during activation remain poorly understood. Our studies have convincingly demonstrated that SREBPs were essential in coordinating TCR signaling with a lipid-anabolic program requisite for rapid membrane biosynthesis and cellular growth. In the absence of SREBP signaling, newly activated CD8+ T cells moved into the G1 phase of the cell cycle but were unable to grow, which resulted in a lower proliferative capacity and considerably attenuated antiviral immune responses. Notably, we traced that defect in growth and proliferation to an insufficiency in cholesterol. Our data support a model in which an intracellular cholesterol pool, probably in the ER (the site of lipid biosynthesis), serves as a metabolic signal that conveys the fitness of blasting T cells. Consequently, the SREBP gene program facilitates the transition of mitogen-stimulated T cells through a critical metabolic checkpoint during blastogenesis. These signals ensure the coordination of cellular metabolism with the cell cycle. Simply put, without sufficient cholesterol, effector T cells are unable to grow in response to conventional antigenic stimulation.

One unexpected result of our study was the finding that SREBP activity was dispensable for the homeostatic proliferation of CD8+ T cells. SREBPs are important in regulating the proliferative capacity and survival of cancer cells, although, albeit through poorly described and disparate mechanisms. The influence of SREBPs on the proliferation of primary cells is even less well understood. Our studies of conventional, antigen-driven proliferation and antiviral immunity supported the proposal that SREBPs influence rapid cellular growth. Nevertheless, our observation that Scap-deficient T cells had no observable defect in homeostatic proliferation provided evidence of differences in requirements for SREBP signaling during proliferation. Thus, the emerging idea that engagement of the SREBP pathway is universally required for cellular proliferation and survival is probably incorrect. Alternatively, we propose that SREBP signaling is necessary under circumstances in which cells require the rapid addition of biomass rather than proliferation itself.

An interrelated and important idea centers on the observation that effector CD8+ T cells and memory CD8+ T cells use distinct metabolic programs. The generation of effector CD8+ T cells seems to be favored by glycolytic and anabolic programs, whereas the generation of memory T cells is enhanced under heightened oxidative phosphorylation and catabolism. Our metabolic studies indicated that SREBP signaling was required for a CD8+ T cell to fully acquire a glycolytic phenotype. One possibility is that inhibition of SREBPs in responding T cells could result in 'preferential' enrichment for memory at the expense of effector-cell generation. Published studies have indicated that moderate inhibition of mTOR signaling with a low dose of rapamycin favors the generation of memory CD8+ T cells, while higher doses promote effector function. Other studies have indicated that mTOR signaling controls cellular metabolism through the HIF and SREBP transcription factors, and our data presented here indicated that the SREBP in CD8+ T cells was sensitive to rapamycin during T cell activation. A logical prediction of such studies is that a low dose of rapamycin would favor the development of CD8+ memory cells through its effects on SREBP activity.

Additional studies will be needed to rigorously test that prediction. Nevertheless, our data raise the possibility that the generation of memory cells and effector cells could be uncoupled by manipulation of sterol metabolism. In conclusion, our studies have delineated a role for SREBPs in controlling conventional, antigen-driven clonal expansion but not homeostatic proliferation. These observations could have implications for the development of new therapeutic strategies that target this pathway to control unwanted effector T cell proliferation without perturbing homeostasis or development.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** GEO: microarray data, GSE44261, GSM1081398, GSM1081399, GSM1081400, GSM1081401, GSM1081402, GSM1081403, GSM1081404, GSM1081405, GSM1081406, GSM1081407, GSM1081408 and GSM1081409.

**Note:** Supplementary information is available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

Y.K. designed, did and analyzed most of the experiments and wrote the manuscript; H.E., M.B.H., L.V., K.J.W., J.P.A., R.N.M., E.K. and E.B.W. did experiments and analyzed data; T.F.O. provided materials and intellectual input; and S.J.B. provided overall coordination for the conception, design and supervision of the study and wrote the manuscript (with input from T.F.O., T.G.G., K.R. and D.G.B.).

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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**ONLINE METHODS**

**Mice.** Scapfl/fl mice17 and C57/BL6 mice were from the Jackson Laboratory. Cdh4-Cre mice were from Taconic. Scapfl/fl mice were crossed with Cdh4-Cre mice to generate Scapfl/fl;Cdh4-Cre mice (called Scap0/0 here). Their Scap0/0 Cdh4-Cre littermates were used as controls in all experiments with Scap0/0 Cdh4-Cre mice. All mice were maintained in pathogen-free facilities of the University of California, Los Angeles. All experiments on mice and tissues collected from mice were in strict accordance with University of California, Los Angeles policy on the humane and ethical treatment of animals.

**Cell culture and reagents.** Cells were cultured in IMDM supplemented with 10% heat inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 50 µM 2-mercaptoethanol. Total T cells or CD8+ T cells were purified with negative enrichment kit (STEMCELL Technologies). T cells were cultured in 96-well plates coated with 5 µg/ml of anti-CD3 (2C11; Bio X Cell) alone or in combination with 2 µg/ml of soluble anti-CD28 (37.51; Bio X Cell) or 100 IU/ml of human IL-2. Alternatively, cells were cultured with 50 ng/ml of PMA (phorbol 12-myristate 13-acetate; EMD Chemicals) alone or in combination with 500 ng/ml of ionomycin (Sigma-Aldrich). For proximal TCR signaling analysis, splenocytes were cultured with 1 µg/ml of soluble anti-CD3. In some experiments, cells were cultured with following inhibitors for 30 min before activation: G69983 (Sigma-Aldrich), Ly294002 (EMD Chemicals), rapamycin (EMD Chemicals) and 25-hydroxycholesterol (Sigma-Aldrich).

**Viral transduction.** Transduced forms of SREBP1a or SREBP2 (Supplementary Fig. 1c) were cloned into the retroviral vector MIGR1 (a gift from Y. Tone and M. Tone). Virions were produced with the 293T human embryonic kidney cell line by transfection with Lipofectamine 2000 (Invitrogen). Purified naive T cells were stimulated in six-well plates (at a density of 2 x 10⁶ cells per well) precoated with 5 µg/ml of anti-CD3 supplemented with 2 µg/ml of anti-CD28. At 24 h, cells were transduced by centrifugation at 1,200 r.p.m. for 90 min at 37 °C in viral medium plus 8 µg/ml of polybrene and 100 IU/ml of IL-2. The medium was then replaced with complete IMDM supplemented with 100 IU/ml of IL-2 and cells were incubated for additional 24 h. Control cells were subjected to the same stimulation and centrifugation as transduced cells were but without virus. After a total of 48 h of stimulation, cells were collected and transduction efficiencies were evaluated by flow cytometry analysis of the expression of green fluorescent protein.

**RNA-mediated interference.** Purified T cells were transected with non-targeting siRNA or SREBP1- or SREBP2-specific siRNA (all from Dharmacon) with Nucleofector according to manufacturer’s protocol (Lonza). Transfected cells were allowed to ‘rest’ for 18 h at 37 °C and then stimulated for 5 h with PMA and ionomycin.

**Quantitative real-time PCR analysis.** RNA was isolated with TRIzol (Invitrogen), then cDNA was synthesized with (S)cript cDNA Synthesis Kit (BIO-RAD). SYBR Green I Master mix (Roche) and a LightCycler 480 (Roche) DNA polymerase. Primers were designed using Primer Express 2.0 software and primer sequences are provided in Supplementary Table 2.

**Flow cytometry.** The membrane-impermeable DNA-intercalating dye 7-AAD (7-amino-actinomycin D) was from BD Biosciences. Anti-mouse CD4 (RM4-5), anti-CD8 (53-6.7), anti-CD25 (PC61.5) and anti-Thy-1.2 (53-2.1) were from BioXCell. Anti-CD4 (IM7), anti-INF-γ (XMG1.2) and antibody to tumor-necrosis factor (MP6-XT22) were from BioLegend. Staining kits for PMA and ionomycin, 6(0)-carboxyfluorescein diacetate succinimidyl ester), ER-Tracker Blue-White Fluorescent Dye), iNOS (B6H12/1; BioLegend) and antibody to cyclin A2 (clone SC-64918) were from Santa Cruz Biotechnology. Anti-HIF-1α (1:0006421) was from Cayman Chemical. Antibody to Akt phosphorylated at Ser473 (D9E), anti-S6 (54D2), antibody to p65 phosphorylated at Ser536 and Ser535 (p-p65; 2552), anti–c–c–cytin D2 (D5229), anti–c–c–cytin D3 (DCS22), anti–c–myc (9402), antibody to p requirements of the experiment. Data were collected on an Agilent 5975C MSD connected to an Agilent 7890 Gas Chromatograph with the Phenomenex ZB-MR-1 column. The cholesterol was run in split mode (1 µl at a dilution of 1:5) with the following settings: inlet temperature, 250 °C; split ratio, 30:1; and column temperature, 140 °C.

**Immunoblot analysis.** Whole-cell extracts were produced from equivalent number of cells in each sample with RIPA buffer (50mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP40, 0.5% deoxycholate and 0.1% SDS) supplemented with calcyxin A (Cell Signaling) and protease inhibitor ‘cocktail’ (Sigma–Aldrich). Samples were separated by electrophoresis through a 4–12% Bis-Tris gel and were transferred to nitrocellulose. Anti-Akt1 (5C10) and anti-actin (I-19) were from Santa Cruz Biotechnology. Anti-HIF-1α (1:0006421) was from Cayman Chemical. Antibody to Akt phosphorylated at Ser473 (D9E), anti-S6 (54D2), antibody to p65 phosphorylated at Ser536 and Ser535 (p-p65; 2552), anti–c–c–cytin D2 (D5229), anti–c–c–cytin D3 (DCS22), anti–c–myc (9402), antibody to p65 phosphorylated at Tyr399 (9E5), antibody to Lck phosphorylated at Tyr305 (2751), anti-Lat (9166), antibody to Lat phosphorylated at Tyr191 (3584), anti-p44/42 (137F5), antibody to p44/42 phosphorylated at Thr202/Tyr204 (D13.14.4E), anit-AMPK (F6), antibody to AMPK phosphorylated at Thr172 (40H9) and antibody to acetyl-CoA carboxylase phosphorylated at Ser79 (5661) were from Cell Signaling.

**ChIP analysis.** The method for this has been described34. Cells (2 x 10⁶) were fixed and sonicated. Pre cleared lysates were incubated overnight at 4 °C with polyclonal antibody to SREBP1 or SREBP2 (a gift from T. Osborne) or control rabbit IgG (2729; Cell Signaling). Immunocomplexes were collected and real-time PCR was done as described above (primer sequences, Supplementary Table 2).

**Gene-expression analysis.** RNA was isolated from CD8+ T cells from Scap−/− deficient mice and their control littermates and was hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 Array. Genes were filtered for differences in expression with the criterion of a P value of less than 0.001 (analysis of variance). For heat-map display, genes were on the mean, normalized and clustered with a Pearson correlation coefficient metric and pairwise complete linkage. Pathway-enrichment analysis was done with the DAVID database20,21. Analysis of transcription factors was generated through the use of IPA software (Ingenuity Systems).

**Metabolic assays.** Cellular ATP was measured from the same number of live cells in each sample with an ATP-determination kit according to the manufacturer’s protocol (Molecular Probes). Background values measured with buffer without cells were subtracted from the values of each cell sample. For mitochondrial mass, cells were incubated for 30 min at 37 °C with 50 nM MitoTracker Green (Molecular Probes). The oxygen-consumption rate and extracellular acidification rate were measured with an XF24 analyzer according to the manufacturer’s recommendations (Seahorse Bioscience)35. Mixing, waiting and measure times were 2, 2 and 4 min, respectively. Test compounds (Sigma) were injected during the assay at the following final concentrations: 0.2 µM oligomycin, 1 µM FCCP and 0.75 µM rotenone–myxothiazol. The concentration of glucose, glutamine and lactate in culture medium was measured with a Bioprofile analyzer (Nova Biomedical).

**Cholesterol loading.** A complex of cholesterol and methyl–β–cyclodextrin containing 40 mg of cholesterol per gram was purchased from Sigma (C4951). Treatment concentration was based on cholesterol weight. Cells were activated for the appropriate time with or without cholesterol–methyl–β–cyclodextrin in 10% FBS.

**Gas chromatography–mass spectrometry.** Purified T cells were used immediately after collection or after 24 h of activation with plate-bound anti-CD3 in combination with soluble anti-CD28 and were washed twice with PBS. Extraction and analysis of fatty acids were done as described36 with the modifications described below. Triheptadecanoin (T-155; Nu-Chek Prep) was used as the internal standard for fatty acids. Ergosterol (17130; Sigma) was used as the internal standard for cholesterol. Internal standards were added to TRIzol. Cholesterol was extracted from organic phase with petroleum ether after saponification but before acidification and fatty-acid extraction. Extracted cholesterol was trimethylsilylated with BSTFA and TMCS (33155-U; Sigma). The concentration of glucose, glutamine and lactate in culture medium was measured with a Bioprofile analyzer (Nova Biomedical).
flow rate, 1.2 ml/min; transfer line, 280 °C; mass spectrometry (MS) quadrupole, 150 °C; MS source, 230 °C; oven set at 230 °C initially, ramped to 280 °C at a rate of 10 °C/min with a hold for 18 min and then ramped to 320 °C at a rate of 20 °C/min with a hold for 5 min (30 min total run time). The fatty acids (FAMES) were run in split mode (1 µl at a dilution of 1:6) with the following settings: inlet temperature, 250 °C; flow rate, 2 ml/min; transfer line 280 °C; MS quadrupole, 150 °C; MS source, 230 °C; oven set at 172 °C initially, ramped to 230 °C at a rate of 2 °C/min and then ramped to 320 °C at a rate of 30 °C/min with a hold for 4 min (36 min total run time). Quantification of the area under the curve for selected ions was done with Chemstation software.

LCMV infection, major histocompatibility complex class I tetramer and intracellular cytokine staining. Mice were infected intraperitoneally with 2 × 10^4 plaque-forming units of LCMV, Armstrong strain. Viral stocks were prepared and viral titers were quantified as described. Splenocytes were stained immediately after isolation with tetramers of H-2D^K and gp33 (amino acids 33–41 of LCMV glycoprotein) or H-2D^K and NP396 (amino acids 396–404 of LCMV nucleoprotein) and for surface expression of CD8. Tetramers of major histocompatibility complex were obtained from the Tetramer Core Facility of the US National Institutes of Health. For analysis of cytokine expression, splenocytes were stimulated for 5 h with 2 µg/ml of the major histocompatibility complex class I–restricted LCMV gp33 or gp33 peptide in the presence of 50 U/ml recombinant mouse IL-2 (R&D Systems) and 50 U/ml brefeldin A (Sigma-Aldrich). Cells were stained for surface expression of CD8, then fixed, permeabilized and stained with anti–tumor-necrosis factor α and anti-interferon-γ (both identified above). Flow cytometry was done with an LSR II or a FACSVersa (Becton Dickinson) and data were analyzed with FlowJo software (Treestar).

Adoptive cell transfer. CD8^+ T cells or total T cells were purified as described above from Scap^fl/fl and their wild-type littermates or Thy-1.1^+ mice spleens. Purified cells were labeled with CFSE (carboxyfluorescein diacetate succinimidyl ester), and 1 × 10^6 to 5 × 10^6 cells per mouse were injected retro-orbitally into a RAG-2-deficient host or a host irradiated 1 d earlier with 600 rads. After 6 d, host spleen and lymph node cells were analyzed by flow cytometry. For gene-expression analysis, Thy-1.1^+ T cells were sorted and real-time PCR was done as described above.

Immunization with OVA peptide in incomplete Freund’s adjuvant. At 48 h hours after the transfer of OT-I cells, mice were either left unimmunized or immunized with total of 300 µg of OVA peptide mixed in incomplete Freund’s adjuvant into three sites in upper back and three sites in the lower back. Spleen and lymph nodes were collected 24 h later and CD3^+CD8^+ cells were analyzed by flow cytometry.

BrdU labeling. A BrdU flow kit from BD Bioscience was used for these analyses. BrdU (2 mg/mouse) was injected intraperitoneally into Scap^fl/fl mice and their littermates. Peripheral blood was collected twice a week and mononuclear cells were stained and analyzed on flow cytometry according to the manufacturer’s protocol (BD Biosciences).

Statistical analysis. Statistical significance was determined with the two-tailed unpaired Student’s t-test unless otherwise indicated.

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