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# Regulation and function of mTOR signalling in T cell fate decision

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## Abstract

The evolutionary conserved kinase mTOR couples cell growth and metabolism to environmental inputs in eukaryotes. T cells depend on mTOR signalling to integrate immune signals and metabolic cues for their proper maintenance and activation. Under steady-state conditions, mTOR is actively controlled by multiple inhibitory mechanisms, and this enforces normal T cell homeostasis. Antigen recognition by naïve $CD4^+$ and $CD8^+$ T cells triggers mTOR activation, which in turn programs their differentiation into functionally distinct lineages. This Review focuses on the signalling mechanisms of mTOR in T cell homeostatic and functional fates and therapeutic implications of targeting mTOR in T cells.

The mammalian target of rapamycin (mTOR; now officially known as the mechanistic target of rapamycin), a conserved serine/threonine kinase, is a central regulator of cell growth and metabolism[1]. mTOR senses and integrates diverse environmental signals including nutrients and growth factors, many of which deliver the inputs to the PI3K–AKT pathways that ultimately activate mTOR. mTOR exists in two multiprotein complexes in metazoans. mTOR complex 1 (mTORC1) contains the scaffolding protein, regulatory associated protein of mTOR (RAPTOR), and is sensitive to the immunosuppressant rapamycin (FIG. 1a). mTOR complex 2 (mTORC2) has a distinct scaffolding protein, rapamycin-insensitive companion of TOR (RICTOR), and is relatively resistant to rapamycin except under prolonged period of treatment. Aberrantly elevated mTOR activity is frequently associated with human malignancies, and consequently mTOR has been the subject of extensive investigation in cancer biology[1]. However, emerging evidence highlights a critical role of mTOR signalling in both the innate and adaptive immune systems.

The outcome of an adaptive immune response depends on the sensing of antigenic and inflammatory signals by T cells. T cells have evolved to perceive these immune stimuli and further coordinate them with diverse environmental and metabolic cues through the evolutionarily conserved mTOR pathway. Thus, mTOR endows T cells with the ability to properly integrate a multitude of signals to dictate the outcome of adaptive immunity. Whereas the first major function ascribed to mTOR in T cells was the promotion of cell cycle progression, more recent studies have established mTOR signalling as a fundamental determinant of cell fate decision both under steady-state and following cognate antigen recognition. mTOR likely affects these diverse processes in T cells by serving as a signalling node to coordinately regulate immune receptor signalling pathways, metabolic programmes

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and migratory activity. As a number of excellent reviews have covered the immune functions of mTOR[2–4], this Review mainly focuses on the most recent genetic studies that have identified new roles for mTOR signalling in T cell fate decision, and on the therapeutic implications of modulating mTOR functions in T cells. Following the hierarchy of signal transduction, first the extracellular inputs that feed into the mTOR pathway in T cells are addressed. Then the mechanisms through which negative and positive components of mTOR signalling impinge upon cell fate decision in T cells are described, with a special focus on T cell homeostasis and T helper cell differentiation (Table 1). Finally, mTOR downstream effector pathways involved in T cell metabolism, and the implications of targeting mTOR and metabolic pathways for disease therapeutics are discussed.

## Upstream input signals to mTOR

In T cells, mTOR senses three broad categories of instructive signals. The first is the immune activation signals transduced from dendritic cells (DCs), including antigens, co-stimulation and inflammatory cytokines, collectively known as signals 1–3, that are essential to direct proper T cell activation and differentiation[5]. The other two instructive signals are mediated by environmental stimuli such as growth and immunomodulatory factors, and metabolic cues derived mainly from nutrients (FIG. 1a). Whereas the immune activation signals are unique to T cells, the environmental and metabolic stimuli act on all eukaryotic cells. Many of the upstream signals activate mTORC1 through the GTP-loaded form of the small GTPase RHEB (FIG. 1a). RHEB is tightly regulated by the tuberous sclerosis 1 (TSC1)–TSC2 complex, which, through its GTPase-activating protein (GAP) activity toward RHEB, inactivates RHEB and mTORC1. The TSC complex serves as a molecular switch to integrate upstream signals, and in particular the positive and negative signals transduced from PI3K–AKT and AMP-activated protein kinase (AMPK) pathways, respectively[1]. RHEB deficiency in T cells blunts mTORC1 activation in response to T cell receptor (TCR) stimulation[6], whereas loss of TSC1 disrupts the entire TSC complex and enhances basal and TCR-induced mTORC1 activity[7–9]. These results highlight a crucial role for the TSC-RHEB axis in T cell responses.

TSC-independent pathways also engage mTORC1, although the importance of these mechanisms in T cells remains to be determined. Activation of mTORC1 in response to amino acids requires the RAG family of GTPases[10, 11], whereas AMPK-mediated direct phosphorylation of RAPTOR inhibits mTORC1 during energy stress[12]. Further, the stress-activated p38β MAP kinase targets different components of mTORC1 to either positively or negatively regulate its activity under different environmental stresses[13, 14]. Upstream pathways that activate mTORC2 are just beginning to be identified, with recent studies showing a role for the ribosome to link PI3K to mTORC2 activation[15, 16]. Moreover, another small GTPase RAC1 binds directly to mTOR to activate mTORC1 and mTORC2, which provides a means to regulate both mTOR complexes simultaneously[17]. The convergence of multiple signals on mTOR suggests that its basic function is a signal integrator.

### Immune signals 1–3

Both mTORC1 and mTORC2 are activated within minutes after TCR stimulation. Magnitude of mTOR activation is directly correlated with the duration of T cell–DC interaction and the dose of the cognate antigen[18, 19]. mTOR activity is further shaped by co-stimulatory signals. CD28 co-stimulation is a classic PI3K–AKT activating signal that in turn upregulates mTOR activity induced by TCR, to facilitate productive T cell activation[20–22]. Another co-stimulatory receptor, OX40, a member of the tumour necrosis factor receptor (TNFR) family, assembles a signalling complex by recruiting PI3K–AKT to augment TCR-dependent AKT signalling[23]. By contrast, the PD1–PDL1 axis, a negative T

cell co-stimulatory pathway, downregulates mTOR signalling to mediate immune tolerance[24].

As compared with TCR-dependent rapid activation of mTOR, the homeostatic cytokine interleukin-7 (IL-7) induces delayed yet sustained PI3K/AKT and mTOR activation. This activation depends on the transcriptional activity of signal transducer and activator of transcription 5 (STAT5), and contributes to IL-7-mediated glucose uptake and tropic effects in T cells[25, 26]. However, increased mTORC1 activity as a result of TSC1 deficiency also impairs IL-7-dependent survival response in naïve T cells[7], suggesting that a defined threshold of mTOR activity is important to mediate IL-7 response. In antigen-stimulated $CD8^+$ T cells, IL-12 enhances and prolongs TCR-dependent mTOR activation, to programme functional maturation of effector cells. Similar to IL-7-stimulated naïve T cells, IL-12-induced mTOR activation in antigen-stimulated $CD8^+$ T cells is indirect and depends on STAT signalling (in this case, STAT4)[27]. In T helper 2 ($T_H2$) and $T_H17$ cells, mTOR is activated by IL-4 and IL-1, respectively, to facilitate cell cycle progression[28, 29]. Furthermore, there is extensive interplay between mTOR and STAT signalling in T cells[6, 30] and other cells[31].

## Leptin receptor

Leptin, an adipose-derived hormone regulating energy metabolism, has long been known to directly regulate T cell proliferation and cytokine production, thereby linking nutritional status and proinflammatory immune responses[32]. More recently, regulatory T ($T_{Reg}$) cells were shown to express leptin and its receptor and contain more abundant mTOR activity relative to conventional T cells[33]. Neutralization of leptin or deletion of leptin receptor considerably diminishes mTOR activity in $T_{Reg}$ cells, suggesting a link between autocrine secretion of leptin and mTOR activation in $T_{Reg}$ cells. The elevated mTOR activity that results from leptin signalling maintains the anergic state of $T_{Reg}$ cells, because transient inhibition of mTOR or neutralization of leptin reverses the hyporesponsiveness of $T_{Reg}$ cells to TCR stimulation, resulting in their robust proliferation[33, 34]. Leptin also activates mTOR in autoreactive $CD4^+$ T cells to promote their survival and mediate autoimmune neuroinflammaton[35]. As the leptin level is directly correlated with nutrient status, the leptin–mTOR axis has been proposed to bridge metabolism and immunity[33].

## S1PR1

Sphingosine 1-phosphate receptor 1 (S1PR1), a G protein-coupled receptor for the bioactive lipid sphingosine 1-phosphate (S1P), is a crucial regulator of T cell egress out of the thymus and secondary lymphoid organs. In $T_{Reg}$ cells, S1PR1 activates AKT and mTOR, and this delivers a cell-intrinsic negative signal to restrain $T_{Reg}$ cell suppressive activity[36]. In conventional $CD4^+$ T cells, S1PR1 is dispensable for immediate mTOR activation but is important to sustain mTOR activity during their differentiation into effector cells[37]. S1PR1-dependent activation of mTOR inhibits the generation of $T_{Reg}$ cells while driving $T_H1$ cell development in a reciprocal manner. These studies identify an S1PR1–mTOR axis that controls $T_{Reg}$ function and T cell lineage choices[36, 37].

## TLRs

Ligation of Toll-like receptors (TLRs) expressed on innate immune cells induces copious amounts of proinflammatory molecules that are important for immediate immune defense. Additionally, both $CD4^+$ and $CD8^+$ T cells express functional TLRs, and recent results suggest a T cell-intrinsic role for TLRs in immune responses. TLR2, via signalling through the adaptor molecule MyD88, activates mTOR and promotes the expression of T-bet in effector $CD8^+$ T cells[38]. Since mTOR is also downstream of TCR signalling, activation of mTOR therefore bridges TCR and TLR signals and promotes effector $CD8^+$ T cell

function[38] and contributes to T cell memory formation[39]. However, MyD88 deficiency in T cells diminishes initial T cell expansion, but not the subsequent generation of the memory population[40]. Further study is required to clarify how TLR2 and MyD88 signalling affects mTOR activation in T cells.

mTOR is also activated by additional extracellular signals in T cells. Insulin induces activation of mTORC1 and promotes mTOR-dependent T-bet expression in antigen-activated $CD8^+$ T cells[27]. Also, NOTCH1 activates AKT–mTOR during early thymic development and relies on AKT-dependent metabolic and trophic effects to regulate thymocyte differentiation and survival[41]. Finally, mTOR is intimately linked to nutrient sensing in all eukaryotic cells[1], and the physiological relevance of metabolic regulation of mTOR is discussed in further details below.

## mTOR in immune homeostasis

T cells develop in the thymus through a step-wise differentiation process. Once mature T cells are released into the periphery, they circulate through the blood and peripheral lymphoid organs in a quiescent state characterized by small cell size and low metabolic activity. Survival of naïve T cells relies on the engagement of TCRs by self-peptide–MHC complexes and on the availability of IL-7, and is further shaped by growth factors and nutrients for metabolic fitness[42, 43]. Given the central role of mTOR as an environmental sensor, it might be predicted that mTOR activity is required for the homeostasis of thymocytes and peripheral T cells. Surprisingly, deletion of the *Mtor* gene after initial thymocyte development (using the CD4-Cre system) had minimal effects on the homeostasis of peripheral T cells under steady state[6], although the requirement of mTOR in early thymic development has yet to be determined. By contrast, several negative regulators of mTOR signalling were found to enforce normal homeostasis of T cells, as manifested by the disrupted development and maintenance in T cells lacking these inhibitory molecules (FIG. 1b).

### PTEN suppresses lymphoma and autoimmunity

Phosphatase and tensin homologue (PTEN) mainly functions as a lipid phosphatase and hydrolyzes phosphatidylinositol-3,4,5-triphosphate, thereby mediating the reverse reaction of PI3K (FIG. 1a). Deletion of PTEN in T cells causes lymphoma that originates in the thymus and is largely driven by c-Myc overexpression[44–49]. $Pten^{-/-}$ thymocytes exhibit markedly elevated AKT and mTOR activities even before transformation[47, 50]. Importantly, tumour development is blocked by mTOR inhibition following rapamycin treatment or deletion of 3-phosphoinositide-dependent protein kinase 1 (PDK1), indicating an crucial role of AKT and mTOR signalling in this process[47, 48]. Somewhat unexpectedly, PTEN deficiency does not overtly disrupt homeostasis of pre-malignant thymocytes, except for some minor defects in cell size and generation of double-positive thymocytes and invariant natural killer T (iNKT) cells[47, 50, 51].

$Pten^{+/-}$ mice develop a late-onset autoimmune disease associated with resistance to FAS-mediated apoptosis[52]. Consistent with this observation, complete loss of PTEN in T cells impairs central tolerance as well as peripheral tolerance[44] and the induction of forkhead box P3 (FOXP3) expression[53]. Recent studies demonstrate that development of autoimmunity in $Pten^{-/-}$ mice can be exclusively mediated by peripheral T cells. Therefore, the two main defects that result from the loss of PTEN function, lymphoma and autoimmunity, are separable and mediated by T cells of distinct developmental stages[49]. Because of the pivotal roles for PTEN in immune homeostasis and function, molecular signals regulating PTEN have received considerable interest. Work from several independent groups has revealed that the microRNA cluster 17–92 represses the expression of PTEN to promote AKT-mTOR

activity in lymphocytes, and consequently controls PTEN-dependent autoimmune and oncogenic functions[54–56].

## TSC1 maintains quiescence of naïve T cells

TSC1 and TSC2 function as an integral complex to stringently control mTORC1 activity (FIG. 1a). We and others have recently found that TSC1-mediated control of mTOR signalling enforces a quiescent programme in naïve T cells by controlling cell size, cell cycle entry and metabolic machinery[7–9]. Abrogation of quiescence predisposes TSC1-deficient T cells to apoptosis, and this results in the loss of conventional T cells and iNKT cells. The remaining *Tsc1*$^{-/-}$ T cells exist in a unique 'semi-activated' (CD44$^{+}$CD122$^{-}$) status *in vivo* and exhibit increased activation, cell cycle entry and cytokine expression after acute TCR stimulation. Despite this, TSC1-deficient mice fail to generate effective anti-bacterial immune responses, even when the excessive apoptosis is largely blocked, which suggests that maintenance of naïve T cell quiescence is important for a productive immune response[7]. The precise mechanism by which TSC1 deficiency dampens the immune response is uncertain but may involve premature activation of the cell cycle and metabolic machineries and transcriptional responses in *Tsc1*$^{-/-}$ naïve cells before they receive proper TCR signals[7]. These studies identify TSC1 as a *bona fide* factor in establishing naïve T cell quiescence to facilitate immune homeostasis and function.

*Tsc1*$^{-/-}$ peripheral T cells exhibit increased mTORC1 but diminished mTORC2 activities[7–9]. Treatment of *Tsc1*$^{-/-}$ mice with rapamycin partly rectifies the altered T cell homeostasis and survival, whereas loss of mTORC2 alone has no apparent effect. These results indicate a crucial contribution for mTORC1 activation to T cell homeostasis[7]. By contrast, in mature thymocytes lacking TSC1, mTORC1 activity is increased but cell survival is not affected[7]. Moreover, although PTEN-deficient T cells upregulate mTORC1 activity, they largely maintain their quiescence prior to tumour development[47, 50], and this suggests a crucial but cell context-dependent effect of mTORC1 on T cell homeostasis. Collectively, these data illustrate that TSC1-dependent active control of mTORC1 is a key checkpoint to enforce quiescence of naïve T cells in the periphery[7–9].

## LKB1 promotes T cell development and survival

Liver kinase B1 (LKB1) phosphorylates and activates AMPK subfamily members in response to energy depletion. Deletion of LKB1 in T cells results in profound defects in multiple compartments, including extensive apoptosis of T cells, impaired thymic selection and dysregulated metabolism and proliferation[57–60]. Despite the well-documented role of the LKB1–AMPK axis in mTORC1 inhibition (FIG. 1a)[61], signalling mechanisms downstream of LKB1 in T cells are not fully understood. The increased mTORC1 activity in *Lkb1*$^{-/-}$ T cells contributes to excessive cytokine production[60], but whether it leads to reduced cell survival or other defects is unclear. Also, although AMPK activity is diminished in *Lkb1*$^{-/-}$ T cells[57–60] and AMPK is activated by TCR stimulation[62], loss of AMPKα1 (the predominant AMPK isoform in T cells) causes only slight disturbance of T cell homeostasis[60, 63]. Therefore, T cell homeostasis likely requires additional AMPK-related kinases regulated by LKB1[61].

Altogether, analyses of T cells deficient in the tumour suppressors PTEN, TSC1 and LKB1 have provided new insight into mechanisms of immune homeostasis. Notably, hematopoietic stem cells (HSCs) lacking these molecules exhibit some analogous defects in the control of proliferation, survival or function as mutant T cells[64–70], suggesting that HSCs and naïve T cells have a common requirement for these pathways for the proper maintenance. Further, the defects in PTEN or TSC1-deficient HSCs are largely responsive to rapamycin correction[64, 66, 67], but LKB1 functions independent of mTOR in stem

cells[68–70]. Therefore, despite the shared ability to inhibit mTOR signalling, these molecules employ distinct pathways for the homeostatic control of HSCs and possibly T cells. Aside from these well characterized mTOR inhibitory molecules, recent studies have identified additional negative regulators of mTOR with important roles in cell signalling and disease regulation, such as DEPTOR[71], SESTRIN[72], and the mTORC1 component PRAS40[73, 74]. The roles of these molecules in T cell-mediated adaptive immunity have not been addressed.

Moreover, mTOR may further interact and crosstalk with transcriptional and immune signalling pathways to mediate T cell homeostasis. In particular, forkhead box O1 (FOXO1), a crucial transcription factor for naïve T cell survival and trafficking[75, 76], is under the stringent control of AKT-mediated phosphorylation and nuclear exclusion and thus could serve as an important downstream target of mTORC2. Consistent with this idea, transcriptional targets of FOXO1 including IL-7 receptor α (IL-7Rα) and trafficking molecules such as CD62L are altered in T cells treated with mTOR- or AKT-inhibitors, or in PTEN-, RICTOR- and PDK1-deficient T cells[75, 77–79]. The functional link and physiological relevance of these interactions remain to be fully defined. Diacylglycerol kinases have also been shown to inhibit TCR-induced mTOR activity by downregulating diacylglycerol-mediated RAS signalling[80], which may contribute to T cell homeostasis and function.

## mTOR in T helper cell differentiation

Early studies on mTOR signalling in T cell responses centered upon the anti-proliferative effect of the inhibitor rapamycin. Analysis of *Mtor*$^{-/-}$ T cells has confirmed a role for mTOR in cell cycle progression, although T cell proliferation is delayed but not abolished[6]. Accumulative evidence, however, highlights a central role for mTOR as a fundamental determinant of cell fate decision of antigen-activated CD4$^+$ and CD8$^+$ T cells. Inhibition of mTOR with rapamycin facilitates induction of anergic and regulatory CD4$^+$ T cells, two crucial components of peripheral tolerance (Box 1), as well as differentiation of memory CD8$^+$ T cells (Box 2). Excellent reviews discuss the role of mTOR signalling in the differentiation of regulatory, effector and memory T cells[4, 81–83], so here I mainly focus on more recent genetic evidence that establishes a crucial role for mTOR in T helper cell differentiation.

### Box 1

#### mTOR in peripheral tolerance

Induced regulatory T ($T_{Reg}$) cells act in synergy with naturally occurring $T_{Reg}$ cells to establish immune tolerance[139]. Inhibition of the mammalian target of rapamycin (mTOR) activity has been shown to induce *de novo* forkhead box P3 (FOXP3) expression[140, 141], or to expand preexisting natural $T_{Reg}$ cells[113]. Conversely, increasing AKT activity, through either deletion of phosphatase and tensin homologue (PTEN) or enforced expression of constitutively active AKT, leads to mTOR-dependent inhibition of induced $T_{Reg}$ cell differentiation[53, 142]. Moreover, several upstream receptors, including programmed cell death ligand 1 (PDL1) and sphingosine 1-phosphate receptor 1 (S1PR1), inhibit the generation of induced $T_{Reg}$ cells through mTOR activation[24, 37]. Two downstream pathways mediate the inhibitory effects of mTOR on induced $T_{Reg}$ cell differentiation (FIG. 2a). First, SMAD3, a key transcription factor downstream of transforming growth factor β (TGF-β) signalling for FOXP3 induction, is antagonized by mTOR signalling in multiple cell types including T cells[6,37,143]. Second, forkhead box O1 (FOXO1) and FOXO3, which induce FOXP3 expression[144–146], are inactivated by AKT-dependent phosphorylation, although how this is controlled by mTOR complex 2 (mTORC2) signalling requires further studies[147]. Interestingly, FOXOs have been shown

to form a complex with SMAD3 to control neuroepithelial and glioblastoma cell proliferation[148], and the integration of SMAD and FOXO signalling by mTOR in T cells will be an interesting topic to explore.

Aside from immune suppression mediated by $T_{Reg}$ cells, another important mechanism of peripheral tolerance is the induction of T cell anergy. T cell anergy is usually induced by TCR (signal 1) alone, in the absence of co-stimulation (signal 2). Among the pathways strongly activated by signal 2 are AKT and mTOR[21, 22]. Indeed, blocking mTOR activity by rapamycin induces T cell anergy *in vitro*, even in the presence of both signals 1 and 2. *In vivo*, mTOR inhibition promotes T cell anergy under conditions that would normally induce active priming, indicating that mTOR has a central role in dictating the decision between T cell activation and anergy[21, 22]. Although rapamycin treatment inhibits the G1 phase of the cell cycle, blocking T cell proliferation alone does not induce anergy[4]. Instead, blocking metabolic pathways necessary for mTOR activation promotes T cell energy, suggesting that mTOR-dependent metabolic control is a key determinant of T cell activation and anergy[21, 22].

**Box 2**

### mTOR in memory CD8$^+$ T cell differentiation

$CD8^+$ T cells constitute an important arm of adaptive immunity due to their response to pathogens by clonal expansion and differentiation into cytotoxic effector cells[149]. During the contraction phase that follows, the majority of effector cells die by apoptosis, while the remaining small subset of antigen-specific T cells develops into long-lived memory T cells. Recent results have revealed a crucial role of mTOR in the lineage decisions between the short-lived effector T cells and memory T cell precursors. Arakai *et al.* described that mTOR modulated memory $CD8^+$ T cell formation in a kinetic and dose-dependent manner[119]. In the model of lymphocytic choriomeningitis virus (LCMV) infection, rapamycin treatment during the T cell expansion phase diminished apoptotic death of effector cells, leading to an increase in the quantity of memory T cells. By contrast, rapamycin treatment during the contraction phase promoted the protective capacity of memory T cells and thus the quality of T cell memory. These effects were dose-dependent as $CD8^+$ T cell expansion was blocked by high dose of rapamycin treatment. Silencing of RAPTOR in T cells recapitulated rapamycin effect, thereby establishing a cell-intrinsic effect of mTORC1 in memory $CD8^+$ T cell formation[119]. However, how mTORC1 is activated by upstream signals in $CD8^+$ T cells is unclear, as it appears to require PI3K[27] but occurs largely independent of AKT[79].

Pearce *et al.* independently identified an inhibitory role of mTOR in memory formation and further linked this function to an upstream regulator, TRAF6[96]. In a model of *Listeria monocytogenes* infection, *Traf6*$^{-/-}$ $CD8^+$ T cells showed normal effector responses but could not develop into memory cells. This defect was associated with the failure to upregulate fatty acid oxidation (FAO), and was rectified by pharmacological activation of AMP-activated protein kinase (AMPK) or inhibition of mTOR[96]. More recently, FAO has been shown to promote mitochondrial respiratory capacity selectively in memory T cells as an important mechanism to promote their survival[150]. In another related study, Rao *et al.* found that rapamycin treatment diminished $CD8^+$ effector function, but promoted memory formation and tumour immunity[27]. mTOR inhibition decreased T-bet levels while inducing EOMES expression as important mechanisms to regulate T cell differentiation[27]. However, deletion of PTEN does not strongly affect $CD8^+$ memory formation[151], and thus the underlying molecular mechanisms remain to be identified.

These studies collectively support a central role for mTOR in dictating effector and memory fate of $CD8^+$ T cells in infectious and tumour immunity.


## Decision making between effector and $T_{Reg}$ cells

Naïve $CD4^+$ T cells respond to antigen stimulation by developing into distinct lineages, including $T_H1$, $T_H2$ and $T_H17$ cells, with specialized properties and effector functions (FIG. 1c), and induced $T_{Reg}$ cells to prevent excessive immune reaction[84]. Powell and colleagues revealed that mTOR promotes the differentiation of effector T cells[6]. *Mtor*$^{-/-}$ T cells show normal TCR-induced activation markers and IL-2 production, but fail to differentiate into $T_H1$, $T_H2$ and $T_H17$ effector cells. Furthermore, they are unable to activate the selective STAT proteins or express the lineage-selective transcription factors (STAT4 and T-bet, STAT6 and GATA3, and STAT3 and RORγt for $T_H1$, $T_H2$ and $T_H17$ cells, respectively). These results identify an indispensable role for mTOR in effector $CD4^+$ T cell differentiation[6]. Conversely, TCR stimulation of *Mtor*$^{-/-}$ T cells results in the spontaneous induction of FOXP3, even in the absence of exogenous cytokines[6]. This phenotype is not observed in either RHEB or RICTOR-deficient T cells, indicating that both mTOR complexes contribute to the inhibition of $T_{Reg}$ cell induction, possibly through distinct downstream mechanisms (FIG. 2a)[6, 30, 77].

## $T_H1$ and $T_H17$ cell differentiation

The two mTOR complexes exert disparate effects on effector T cell differentiation. Deficiency of RHEB, and consequently the loss of mTORC1 activity, largely recapitulate the impaired $T_H1$ and $T_H17$ cell differentiation in *Mtor*$^{-/-}$ T cells, suggesting that mTORC1 mediates mTOR-dependent $T_H1$ and $T_H17$ cell differentiation[30]. *Rheb*$^{-/-}$ T cells express increased levels of suppressor of cytokine signalling 3 (SOCS3), a negative regulator of STAT signalling, and silencing of SOCS3 expression restores $T_H1$ cell differentiation in these cells (FIG. 2b). Thus, mTORC1 signalling promotes $T_H1$ cell differentiation by modulating cytokine signalling[30]. Since mTORC1 activity can also be regulated by upstream signals other than RHEB[12], how such RHEB-independent pathways contribute to T cell differentiation awaits future investigation. Additionally, deficiency of RICTOR and thus loss of mTORC2 activity reduce $T_H1$ cell differentiation through the downregulation of AKT signalling (FIG. 2b)[77], although another independent study shows a dispensable role for mTORC2 in $T_H1$ cell differentiation[30]. How mTOR affects $T_H17$ cell differentiation remains to be fully established, but could partly involve the upregulation of hypoxia-inducible factor 1α (HIF1α) (see below for details)[85, 86].

## $T_H2$ cell differentiation

In contrast to RHEB/mTORC1 signalling, mTORC2 is required for $T_H2$ cell differentiation (FIG. 2c). Two groups have independently demonstrated that loss of RICTOR impairs $T_H2$ cell differentiation *in vitro* and *in vivo*, without appreciably affecting the development of $T_H17$ cells[30, 77]. Lee *et al.* attributed the inability of *Rictor*$^{-/-}$ T cells for $T_H2$ cell differentiation to decreased protein kinase C-theta (PKCθ) activity and nuclear factor-κB (NF-κB)-mediated transcription, as complementation with activated PKCθ restored the $T_H2$ defects[77]. Delgoffe *et al.* described elevated SOCS5 expression in *Rictor*$^{-/-}$ T cells, which accounted for the diminished $T_H2$ response[30]. Collectively, these results establish mTORC2 as a crucial regulator of $T_H2$ cell differentiation[30, 77], and future studies will determine whether PKCθ/NF-κB and SOCS5 act in separate pathways or are components of the same

signalling cascade. By contrast, mTORC1 negatively controls $T_H2$ cell differentiation, as indicated by the increased STAT6 activation and GATA3 expression in *Rheb*$^{-/-}$ T cells (FIG. 2c)[30].

In summary, mTOR dictates cell fate decision between effector and regulatory T cells, with mTORC1 and mTORC2 exerting distinct effects on immune receptor signalling. These effects are further shaped by the metabolic pathways, which will be discussed below. Moreover, loss of mTORC1 or mTORC2 activity impairs T cell proliferation, with a stronger effect observed in *Rheb*$^{-/-}$ T cells[30, 77]. Cell cycle progression is known to be a prerequisite for T cell differentiation, partly by allowing epigenetic remodeling of cytokine loci[87]. It would be informative to examine how mTORC1 and mTORC2 regulate cell cycle progression, and whether this affects epigenetic regulation or lineage differentiation.

## Effector pathways and metabolic regulation

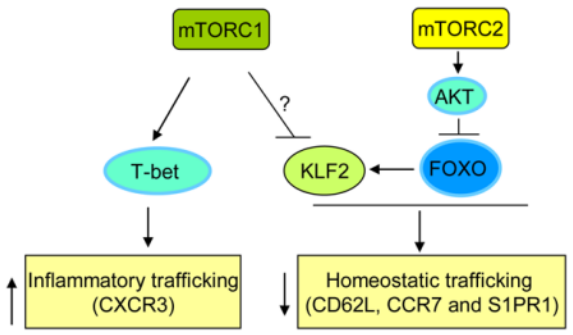
To orchestrate T cell homeostasis and differentiation, mTOR affects several downstream pathways, including those involved in immune receptor signalling, metabolic programming and T cell trafficking (Box 3). Whereas the effects of mTOR on immune receptor signalling molecules, as described above, are easy to appreciate, the relative contribution of mTOR to T cell metabolism and trafficking and how this influences cell fate decision *in vivo* remain under debate[4, 81, 83]. However, evidence is emerging that mTOR serves as a signalling node to regulate both T cell metabolism and migration and further link them to immune signalling and transcriptional networks, to ensure that the metabolic programme and migratory activity match cell fate decision of T cells. In particular, recent studies have revealed exciting new findings on how mTOR-dependent metabolism controls T cell fate, an area with notable therapeutic implications.

### Box 3

#### mTOR in T cell trafficking

Trafficking of naïve and memory T cells requires signals transduced through chemokine receptors and trafficking molecules, such as CC chemokine receptor 7 (CCR7), CD62L and sphingosine 1-phosphate receptor 1 (S1PR1). Antigen-stimulated effector T cells downregulate these surface molecules, but upregulate proinflammatory chemokine receptors and adhesion molecules that endow these cells with increased ability to traffic to sites of inflammation instead of entering secondary lymphoid organs[152]. Therefore, quiescent and activated T cells are characterized by differential migratory activities, and proper regulation of this process is essential for a productive immune response. A role for mTOR in T cell migration was revealed by the observation that rapamycin treatment prevented the downregulation of CCR7, CD62L and S1PR1 in $CD8^+$ T cells responding to TCR or cytokine signals[78]. Rapamycin-treated effector cells tend to migrate to lymph nodes and spleen rather than nonlymphoid tissues[27, 78]. Conversely, deficiency of phosphatase and tensin homologue (PTEN) or tuberous sclerosis 1 (TSC1) is sufficient to downregulate these surface receptors[7, 75, 78]. mTOR-induced effects may involve both mTORC2–AKT–FOXO and AKT–mTORC1 pathways, and depend upon KLF2, a critical transcription factor for CCR7, CD62L and S1PR1 expression[83]. This is further supported by the observations that inhibition of AKT activity or deficiency of 3-phosphoinositide-dependent protein kinase 1 (PDK1) enhances KLF2 expression and the downstream trafficking molecules[79]. In addition to controlling KLF2 expression, mTOR is required for the expression of T-bet[27], which, among many of its downstream effects, directs the expression of proinflammatory chemokine receptors to coordinate T cell migration with effector and regulatory activities[153, 154]. These results highlight that

regulation of T cell migration is an important mechanism by which AKT-mTOR signalling modulates immune function *in vivo*[83].




## Metabolism in T cell activation and differentiation

Naïve T cells utilize a catabolic metabolism where they generate ATP through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. T cell activation markedly elevates uptake and consumption of glucose and glutamine, with a concomitant suppression of fatty acid oxidation (FAO) (FIG. 3a)[43, 88–90]. Among the central regulators of this metabolic reprogramming induced by TCR stimulation are PI3K–AKT signalling and c-Myc, which regulate glucose metabolism and a global metabolic transcriptome, respectively[88, 91, 92]. Additional transcription factors such as estrogen-related receptor-alpha (ERRα) also contribute[93], but HIF1α, which has overlapping functions with c-Myc in cancer cell metabolism[94], is not required for TCR activation-induced metabolic reprogramming[88]. PI3K–AKT pathways signal through mTOR, which further engages an extensive crosstalk with c-Myc in many cellular contexts[95]. In activated T cells, rapamycin inhibits expression of c-Myc and induction of glycolysis[85,88], whereas T cells lacking c-Myc fail to fully activate mTORC1 in response to TCR signals[88]. Consistent with a pivotal role of mTOR in T cell metabolism, *Tsc2*$^{-/-}$ T cells, with constitutive mTOR activity, are highly glycolytic after TCR stimulation[93]. Moreover, mTOR orchestrates the metabolic programme of naïve T cells under steady state, as gene expression programmes for glucose, nucleotide and amino acid metabolism are abnormally upregulated in *Tsc1*$^{-/-}$ naïve T cells that exhibit increased mTORC1 activity[7]. In summary, mTOR has a vital role in orchestrating the metabolic programmes in both naïve and activated T cells.

Recent studies further demonstrate that a metabolic switch to AMPK-dependent FAO is required for the differentiation of CD8$^+$ memory T cells and CD4$^+$ $T_{Reg}$ cells[96, 97], both of which are less anabolic than their effector counterparts[4]. Defective memory formation in *Traf6*$^{-/-}$ CD8$^+$ T cells is associated with the failure to upregulate FAO, and enhancement of FAO by rapamycin or metformin (to activate AMPK) treatment restores the memory formation[96]. Notably, AKT-independent metabolism has been identified in CD8$^+$ T cell responses[79, 83], although such findings do not necessarily exclude a role for mTOR in this process because of a weak effect of AKT inhibition on mTOR activity in CD8$^+$ T cells[79]. In CD4$^+$ T cells, elevated FAO and AMPK activities are associated with differentiation into $T_{Reg}$ but not effector cells, and FAO inhibition prevents rapamycin-induced $T_{Reg}$ cell induction[97]. Therefore, a common component for the differentiation of memory and regulatory T cells is the selective requirement of FAO, in a process reciprocally regulated by mTOR and AMPK.

In contrast to $T_{Reg}$ cells and memory CD8$^+$ T cells, effector T cell differentiation is accompanied by strong upregulation of glycolysis. Pharmacological blocking of mTOR or

glycolysis reduces $T_H1$, $T_H2$ and $T_H17$ cell differentiation[85, 97]. HIF1α is selectively expressed in T cells undergoing $T_H17$ cell differentiation and its induction requires signalling through mTOR[85, 86]. HIF1α is required for mediating glycolysis during $T_H17$ cell differentiation and contributes to the lineage choices between $T_H17$ and induced $T_{Reg}$ cells[85]. Therefore, mTOR-dependent induction of transcription factors c-Myc and HIF1α orchestrates a metabolic checkpoint in TCR-activated and $T_H17$-polarized T cells, respectively[85, 88]. HIF1α has also been shown to exert direct effects to promote degradation of FOXP3 and the transcriptional activity of RORγt[86]. Interestingly, similar to the induction of $T_{Reg}$ cells, $T_H17$ cell differentiation appears to be particularly sensitive to metabolic perturbations. Impairment in $T_H17$ cell differentiation results not only from blockade of glycolysis[85], but also from depletion of selective amino acids[98] and increases in lipid metabolism[99]. As mTORC1 is a key regulator of amino acid and lipid metabolism[100], it will be interesting to examine whether these additional metabolic processes in $T_H17$ cells are regulated by mTOR.

## Sensing and propagating metabolic cues

As a central environmental sensor, mTOR links growth factor signalling and availability of nutrients especially amino acids[1, 101]. This ancient pathway of amino acid metabolism is acquired by the immune system as an important mechanism for immune regulation[102]. In response to $T_{Reg}$ cell-mediated suppression, DCs upregulate the enzymes that consume multiple essential amino acids present in the tissue microenvironment. Consequently, T cells respond to nutrient starvation by downregulation of mTORC1 activity and induction of FOXP3[103]. Although other nutrient sensors such as GCN2 have been identified in T cells[104], mTORC1 appears to have a dominant role in sensing amino acid availability[103, 105]. Consistent with this notion, inhibition of mTOR-dependent amino acid or glucose metabolism attenuates T cell activation and instead induces T cell anergy (Box 1)[21, 22].

Despite these advances, the extent to which mTOR-dependent metabolic pathways regulate T cell differentiation remains a contentious issue[4, 81, 83]. As T cell fate is ultimately manifested as lineage-specific gene expression programme, how do the metabolic intermediates downstream of mTOR activation dictate signalling and transcriptional events? Here I would like to discuss three potential mechanisms (FIG. 3b). First, certain metabolic intermediates, such as reactive oxidative species (ROS) and nicotinamide adenine dinucleotide (NAD) whose production in the metabolic flux depends on mTOR, are now recognized to exert direct signalling activities by serving as substrates or modifiers of enzymes and other regulators, thereby functioning as metabolic checkpoints[106]. Given the recently identified roles of ROS and $NAD^+$-dependent sirtuin 1 in T cell function and differentiation[107, 108], mTOR-dependent metabolic flux may directly engage signalling transduction and crosstalk. Second, nutrient/energetic signals are able to mediate positive and negative feedbacks on mTOR activity itself, which may affect not only cell metabolism but also other mTOR-dependent events such as immune receptor signalling. In particular, activation of mTORC1 by amino acids and the crosstalk with c-Myc upregulate the expression of the amino acid transporter CD98, which establishes a positive loop that amplifies mTOR signalling in T cells[88, 101]. Considering the classic role of mTOR in protein translation, which consumes amino acids, it is perhaps appropriate that mTOR activity is regulated by such a positive loop. Consistent with this notion, inhibition of translation with cycloheximide considerably promotes mTORC1 activity, presumably by elevating the levels of intracellular amino acids[109]. However, excessive mTORC1 activation may deplete cellular ATP and cause energetic stress, which activates AMPK to negatively control mTOR activity[110]. Third, metabolism is intricately linked to cell cycle and apoptotic machineries[111, 112]. T cells with proper metabolism are likely to exhibit preferential survival

and/or expansion, and consequently on a population level, are selected to develop into a particular lineage. In support of this notion, rapamycin differentially affects the proliferation and survival of effector and $T_{Reg}$ cells[113–116]. Future efforts to identify the molecular components orchestrating these processes should provide exciting insight into the interface between metabolism and immunity.

## Therapeutic targeting of mTOR

The immunosuppressive effect of rapamycin dates back to the 1970s, before the identification of its molecular target. Recent studies suggest that blocking mTOR not only mediates immunosuppression in transplant rejections and autoimmune disorders, but also boosts immunity under selective conditions and impacts other aspects of T cell homeostasis and functions.

### mTOR inhibition for immunosuppression

In late 1990s, FDA approved the use of rapamycin to prevent rejection in kidney transplants. A chief mechanism of action for rapamycin is the induction and expansion of $T_{Reg}$ cells and the inhibition of effector T cell differentiation, as described above. This is distinct from other immunosuppressants such as cyclosporine A and FK506 that mainly block $Ca^{2+}$ and calcineurin activation downstream of TCRs, thereby allowing the design of combinatorial therapy of rapamycin and other immunosuppressive agents. This is important because rapamycin monotherapy exerts a rather weak effect to prevent graft rejection, despite its multiple immunomodulatory functions.

The immunosuppressive effect of rapamycin is also apparent in patients and experimental models of systemic autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis, as well as organ-specific autoimmune disorders[117, 118]. Rapamycin likely establishes long-term immune tolerance in these models by expanding $T_{Reg}$ cells and inhibiting effector T cell differentiation and function. Notably, rapamycin also exerts potent effects on DCs and other immune components[2], and the effects of rapamycin *in vivo* might be attributed to these cells, in addition to T cells.

### Rapamycin for vaccine development to boost immunity

Despite the well established immunosuppressive effects of mTOR inhibition, rapamycin and metformin promote the generation of protective T cell memory in the models of infection with lymphocytic choriomeningitis virus (LCMV) and *Listeria monocytogenes*. Mechanistically, this has been associated with the induction of a metabolic switch from glycolysis to FAO in the presence of rapamycin and metformin[96, 119]. The immunostimulatory effect of rapamycin for $CD8^+$ T cell responses has been extended to additional infectious models[120, 121], and anti-tumour immune responses[122, 123]. Therefore, mTOR inhibitors may serve as novel adjuvants for vaccine development against pathogens and tumours. In particular, this strategy of tumour immunotherapy is promising due to its synergy with direct suppression of tumour growth by mTOR inhibitors. Notably, the effect of mTOR inhibition depends on the dose range and kinetics of the treatment. Administration of a very high dose of rapamycin prevents $CD8^+$ T cell expansion, whereas the duration and time points of rapamycin treatment impact the quantity and quality of memory T cell responses[82, 119]. To explore the basis for rapamycin-mediated immunosuppressive and immunostimulatory effects on $CD8^+$ T cell responses, Ferrer *et al.* compared the effects of rapamycin on immune responses elicited by *L. monocytogenes* and by a skin transplant against the same antigen[120]. Treatment with rapamycin augmented antigen-specific T cell responses to the pathogen but not to the transplant. Thus, the environment in which an antigen is presented influences the effects of rapamycin on T cell responses[120].

## T cell malignancy and metabolic dysregulation

A common hematological malignancy is T cell acute lymphoblastic leukemia (T-ALL) that frequently harbors activating NOTCH1 mutations and/or loss-of-function mutations of PTEN. Interestingly, both types of mutations activate mTOR signalling, suggesting a pivotal role for mTOR in T-ALL development. Consistently with this notion, in a PTEN-null T-ALL mouse model, rapamycin was effective to halt T-ALL initiation and development. However, upon rapamycin withdrawal, the majority of PTEN-null mice rapidly became sick and died precipitously. These results indicate that leukemia stem cells and leukemia blasts have differential responses to rapamycin, which could contribute to the limited clinical efficacy of rapamycin in cancer[48].

Chronic inflammation in the adipose tissue has been established as a major mechanism to cause insulin resistance and subsequent development of type 2 diabetes. Although earlier studies mainly implicated macrophages as a major inflammatory infiltrate[124], recent reports identify the recruitment of effector $CD8^+$ T cells and $FOXP3^+$ $T_{Reg}$ cells to the adipose tissue, where they function to exacerbate and ameliorate the inflammation, respectively[125–127]. Given a prominent immunomodulatory role of mTOR in T cells, whether mTOR signalling in T cells contributes to the pathogenesis of metabolic disease will be interesting to explore. This raises the notion that therapeutic targeting of mTOR in metabolic diseases may have a dual effect through the regulation of T cell metabolism and effector function as well as the direct modulation of the function of metabolic tissues (such as adipose tissue, skeletal muscle and liver), in which mTOR signalling has a central role.

## Targeting mTOR: beyond rapamycin

Rapamycin inhibits mTOR through an unusual allosteric mechanism that requires binding to its intracellular partner FKBP12. Whereas rapamycin strongly inhibits the functions of S6 kinases (S6Ks) downstream of mTORC1, it exerts a surprisingly weak effect on the phosphorylation of the other major mTORC1 target, eIF4E-binding proteins (4E-BPs)[128]. Moreover, rapamycin treatment frequently abrogates feedback inhibition of mTORC1 on PI3K–AKT signalling, leading to paradoxical enhancement of AKT activity[129]. The incomplete inhibition on mTORC1 and the reversal of the feedback loop by rapamycin attenuate its therapeutic effects, as reflected by the disappointing therapeutic outcomes of rapamycin and other first-generation mTOR inhibitors in cancer patients[130]. Therefore, selective ATP-competitive mTOR inhibitors, including Torin1 and PP242, were recently developed that completely inhibit mTORC1, including rapamycin-resistant phosphorylation of 4E-BPs[131–133]. As compared with rapamycin, these second-generation inhibitors exert stronger inhibition on mTORC2 and thus are less likely to activate the feedback loop[131–133]. Indeed, these new inhibitors exhibit greater anti-tumour effects than rapamycin[134, 135], and some of them have entered clinical trials as new cancer therapeutics[130]. The immunomodulatory function is anticipated to be extensively investigated in the near future.

Because of the pleiotropic effects of mTOR in T cells, targeting the upstream and downstream components of mTOR signalling, rather than mTOR itself, offers an alternative strategy for added specificity. In particular, targeting mTOR-dependent metabolic pathways is efficacious for modulating T cell-mediated diseases. A classic example is the AMPK-activators metformin and AICAR that block energy-mediated activation of mTORC1, without interfering with the PI3K–AKT-mediated growth factor signalling. Aside from modulating T cell memory as described above[96], the AMPK activators exert both prophylactic and therapeutic effects on EAE by attenuating the severity of clinical disease[136, 137]. Similarly, blocking glycolysis protects mice from autoimmune neuroinflammation mediated by $T_H17$ cells[85]. Moreover, blocking upstream activators of mTOR in T cells, such as S1PR1 and leptin receptor, shows similar effects as rapamycin in

modulating T cell responses in selective models[33, 37]. Further studies of mTOR-dependent signalling axes will provide more opportunities to specifically targeting mTOR-associated pathways.

## Concluding remarks

Cell fate decision mediated by mTOR is of particular importance to T cells because of their unique features: a constant exposure to unpredictable pathogen threats, extensive proliferation following antigenic stimulation, and continuous migration in a variety of tissues. Whereas the recent advances have established mTOR as a fundamental determinant of T cell homeostatic and functional fates, many open questions remain. Further studies are needed to elucidate the upstream signals and downstream effectors that mediate T cell homeostasis and antigen-specific immune responses. Also, we have yet to define the emerging roles of mTOR in certain cell biological processes such as autophagy, a process crucial for T cell homeostasis and function[138]. Much of our current knowledge on the mTOR-controlled signalling networks is derived from the use of *in vitro* systems and cell lines. However, identification of T cell-specific and context-dependent signals promises to provide more insight into regulation of adaptive immunity. From this perspective, the use of sophisticated genetic systems offers the ultimate molecular and cellular specificities to dissect the underlying processes. Moreover, although research in this area has benefited from the use of rapamycin over the last two decades, the development and application of more potent and selective inhibitors of mTOR are essential to advance the research and clinical application. The continued expansion of our understanding of mTOR signalling will manifest legitimate therapeutic opportunities for a number of T cell-mediated disorders.

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## Glossary

| | |
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| **Metabolism** | Intracellular chemical reactions that convert nutrients and endogenous molecules into energy and biomass (proteins, nuclear acids and lipids). Naïve T cells utilize a catabolic metabolism where they use glucose, fatty acids, and amino acids for ATP generation through the TCA cycle and oxidative phosphorylation. Upon antigen stimulation, the bioenergetic demands of a T cell increase dramatically over the resting state and transition into anabolic metabolism mediated by glycolysis and glutaminolysis |
| **AMP-activated protein kinase (AMPK)** | A group of serine/threonine kinases that is activated in response to energy depletion. AMPK is an important activator of fatty acid oxidation and a potent inhibitor of mTORC1 |
| **FOXP3$^+$ $T_{Reg}$ cells** | A critical subset of CD4$^+$ T cells for the maintenance of immune tolerance. Although $T_{reg}$ cells mainly develop in the thymus as a separate lineage of CD4$^+$ T cells, known as naturally occurring $T_{Reg}$ cells, a second subset of $T_{reg}$ cells arises *de novo* from conventional T cells in the periphery upon antigen stimulation in the presence of TGF-β (induced $T_{Reg}$). Invariant natural killer T |

| | |
|---|---|
| | (iNKT) cells. A subset of immune cells that shares properties with both T cells and natural killer cells |
| **Central tolerance** | A process that eliminates self-reactive lymphocytes during their ontogeny. For T cells, this occurs mainly through clonal deletion in the thymus |
| **Peripheral tolerance** | A process that downregulates activation of self-reactive T cells in secondary lymphoid organs (i.e., lymph nodes and spleen). Two of the most important mechanisms are the suppression by $T_{reg}$ cells and induction of T cell anergy |
| **Catabolic metabolism** | The breakdown of complex substances into simpler ones, often accompanied by ATP production. Examples include the oxidation of fatty acids and amino acids |
| **Oxidative phosphorylation** | A metabolic pathway that produces ATP from the oxidation of nutrients and transfer of electrons in a two-step process in mitochondria. The first reaction involves the conversion of intermediate molecules (pyruvate and fatty acids) to acetyl coenzyme A (acetyl-CoA) and the degradation of acetyl-CoA to carbon dioxide in the tricarboxylic-acid (TCA) cycle, yielding free electrons that are carried by NADH and $FADH_2$. The second reaction involves the transfer of electrons from NADH and $FADH_2$ to the electron-transport chain (ETC), resulting in the movement of protons out of the mitochondrial matrix and the generation of electrochemical potential for ATP synthesis |
| **Fatty acid oxidation (FAO)** | An important metabolic process to derive energy by mobilization and oxidation of fatty acids, mainly in the mitochondrion matrix. FAO is positively and negatively regulated by AMPK and mTOR, respectively |
| **T cell anergy** | A state of T cell unresponsiveness to antigen stimulation by failing to proliferate and produce IL-2 |
| **Autophagy** | A recycling process in which the cell degrades cytoplasmic organelles and proteins in lysosomes |

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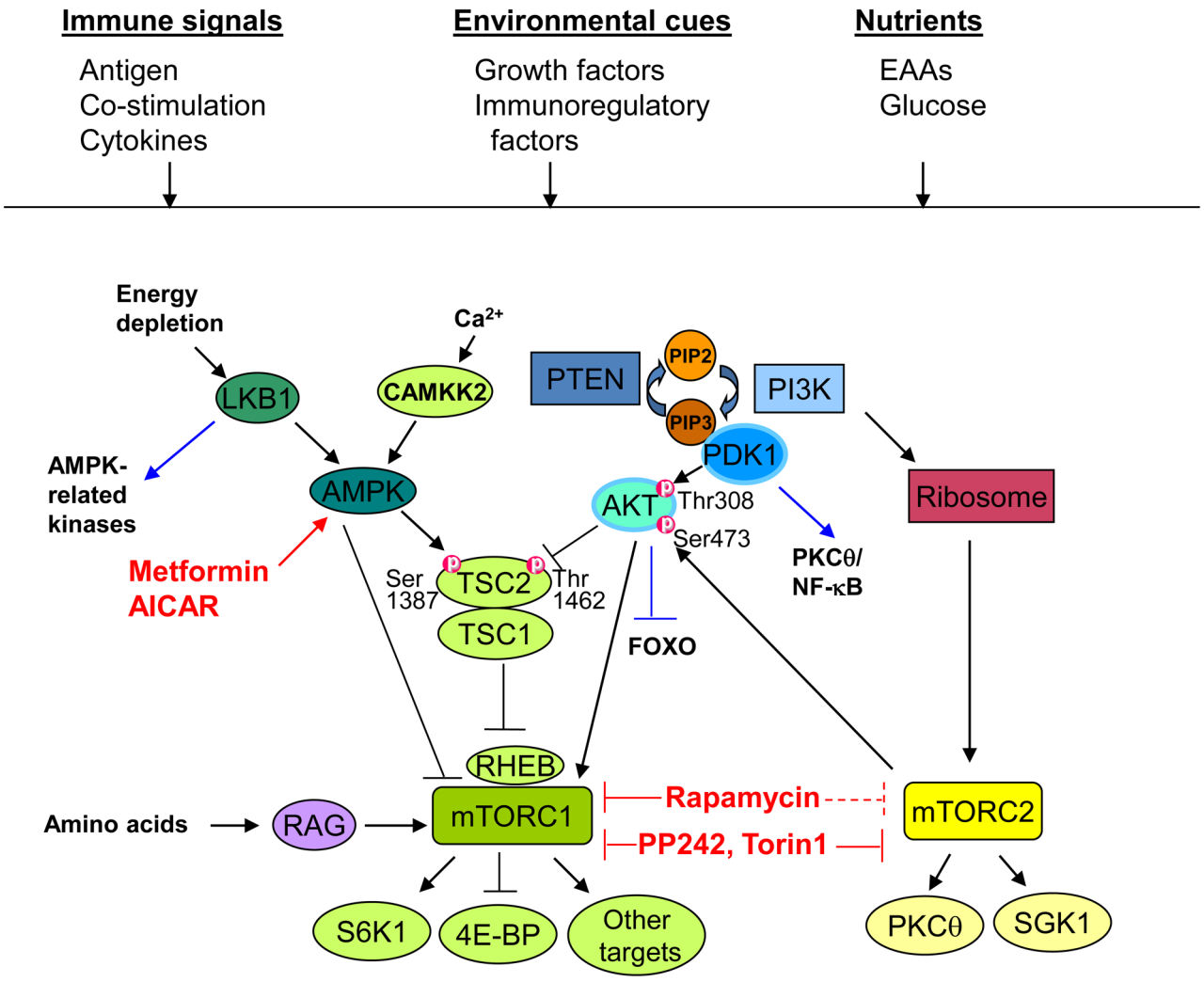
## Biography

Hongbo Chi received his Ph.D. from the University of Rochester, and his postdoctoral training from Yale University School of Medicine. In 2007, he started his independent research program at St. Jude Children’s Research Hospital, where he is currently an Associate Member in the Department of Immunology. His research focuses on the signalling mechanisms, in particular the kinase pathways mediated by mTOR and MAPK, in the immune system.

a



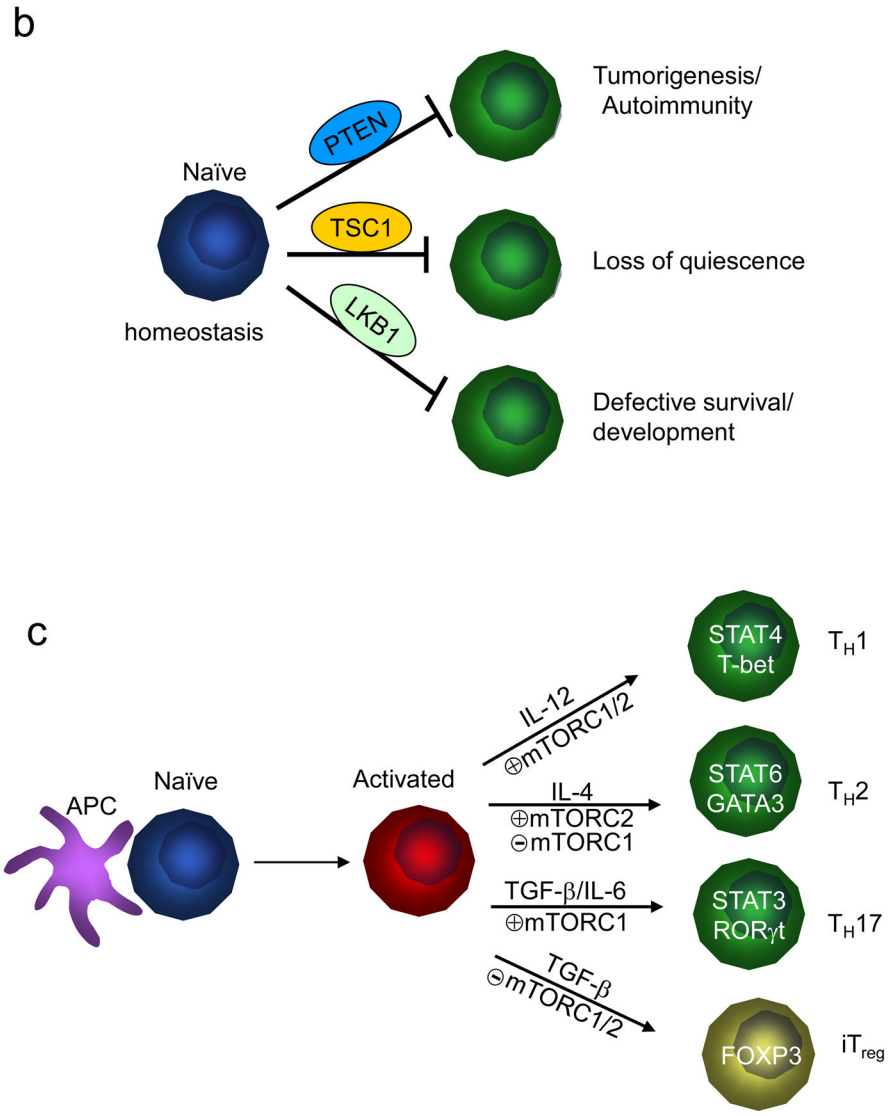


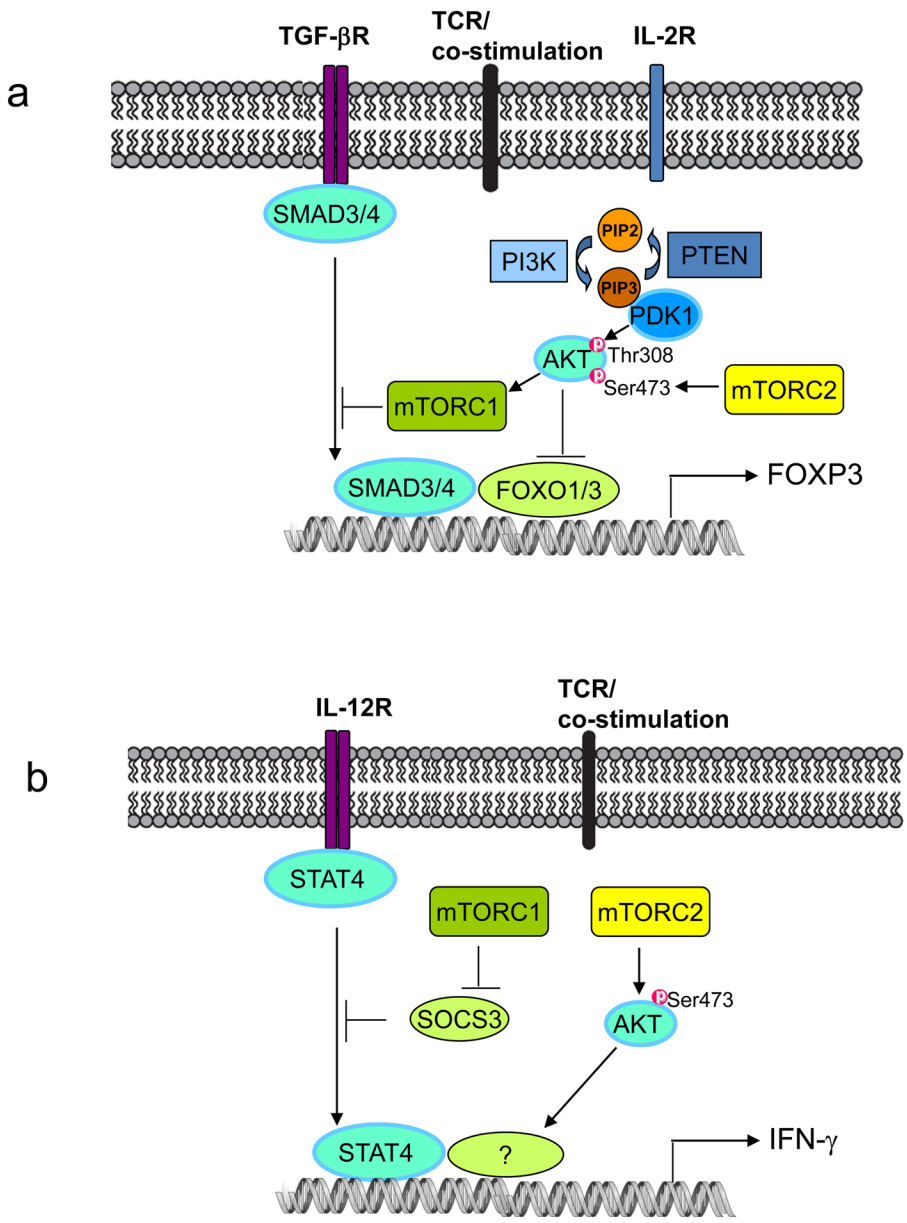

**Figure 1. Regulation and function of mTOR signalling pathways in T cells**

**(a) Components of mTOR signalling.** In T cells, mammalian target of rapamycin (mTOR) can be activated by multiple signals, including the conventionally defined signals 1–3 (antigenic stimulation, co-stimulation and cytokines), growth factors and immunomodulatory factors (such as leptin and sphingosine 1-phosphate (S1P)), and nutrients. The tuberous sclerosis 1 (TSC1)–TSC2 complex integrates signals from phosphoinositide 3-kinase (PI3K)–AKT and liver kinase B1 (LKB1)–AMP-activated protein kinase (AMPK) pathways, and this is mediated by reciprocal regulation of TSC2 activity through AKT-dependent Thr1462 and AMPK-dependent Ser1387 phosphorylation. Upon antigen stimulation, TSC is inactivated by T cell receptor (TCR) signals to mediate mTORC1 activation, but TSC function is maintained in naïve T cells to keep mTOR complex 1 (mTORC1) in check. Additionally, AKT and AMPK can directly modulate mTORC1 functions independently of TSC and RAS homologue enriched in brain (RHEB), and amino acids activate mTORC1 via the RAG family of small GTPases. mTORC1 is best known for its function to promote translation initiation and protein synthesis by directly phosphorylating the substrates S6 kinases (S6Ks) and eIF4E-binding proteins (4E-BPs).

Additional mTORC1 targets include the regulatory proteins in cell signalling, metabolism and autophagy. mTORC2 is important for full activation of AKT by inducing Ser473 phosphorylation (thus AKT can be both upstream of mTORC1 and downstream of mTORC2) and for phosphorylation of various protein kinase C (PKC) isoforms including the activation of PKCθ/NF-κB in T cells and serum and glucocorticoid-inducible kinase 1 (SGK1). EAA, essential amino acid; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate.

**(b) Control of T cell homeostasis by active inhibition of mTOR.** Under steady state, negative inhibitory molecules for mTOR actively maintain the homeostasis of T cells in the thymus and periphery by preventing them from engaging alternative paths. Although phosphatase and tensin homologue (PTEN), TSC1 and LKB1 have shared capacity to inactivate mTOR, they exert distinct effects to enforce T cell homeostasis.

**(c) Role of mTORC1 and mTORC2 in functional differentiation of $CD4^+$ T cells.** Following antigen stimulation, mTOR signalling promotes differentiation of T helper 1 ($T_H1$), $T_H2$ and $T_H17$ effector cells, and inhibits the induction of regulatory T ($T_{Reg}$) cells.



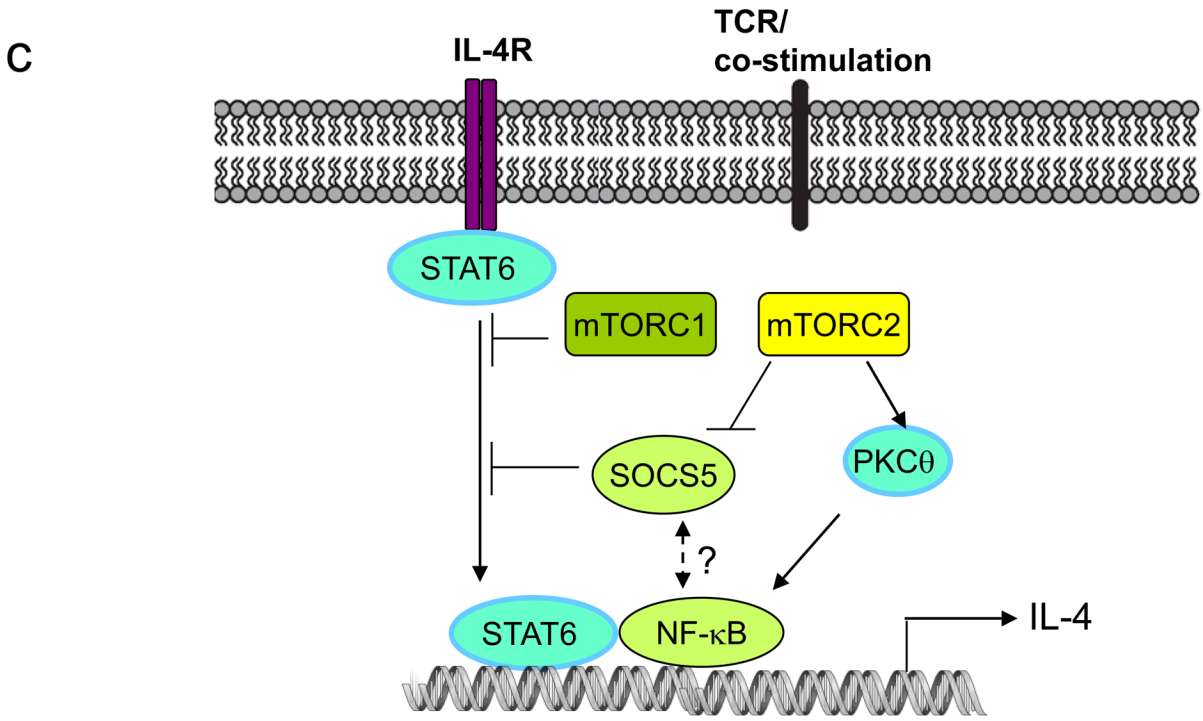



**Figure 2. mTOR-dependent signalling in CD4$^+$ T cell differentiation**

**(a) Differentiation of induced regulatory T ($T_{Reg}$) cells.** Induction of forkhead box P3 (FOXP3) expression depends on the transcription factors SMAD3, SMAD4, FOXO1 and FOXO3. mTOR inhibits induced $T_{Reg}$ cell differentiation by antagonizing the function of SMAD3 and SMAD4 downstream of transforming growth factor β (TGF-β) signalling, and by inducing nuclear exclusion of FOXO1 and FOXO3. These two effects are likely mediated by mTOR complex 1 (mTORC1) and mTORC2, respectively.

**(b) Differentiation of T helper 1 ($T_H1$) cells.** mTORC1 inhibits induction of suppressor of cytokine signalling 3 (SOCS3), a crucial negative regulator of signal transducer and activator of transcription 4 (STAT4), to promote interleukin-12 (IL-12) signalling and $T_H1$ cell differentiation. Additionally, mTORC2 is required for activation of AKT that also contributes to interferon γ (IFN-γ) production.

**(c) Differentiation of $T_H2$ cells.** mTORC2 promotes $T_H2$ cell differentiation via two mechanisms: by preventing expression of SOCS5, a negative regulator of IL-4 and STAT6 signalling, and by activating protein kinase C-theta (PKCθ) signalling and nuclear factor-κB (NF-κB)-mediated transcription. By contrast, mTORC1 negatively constrains STAT6 signalling and $T_H2$ cell differentiation.

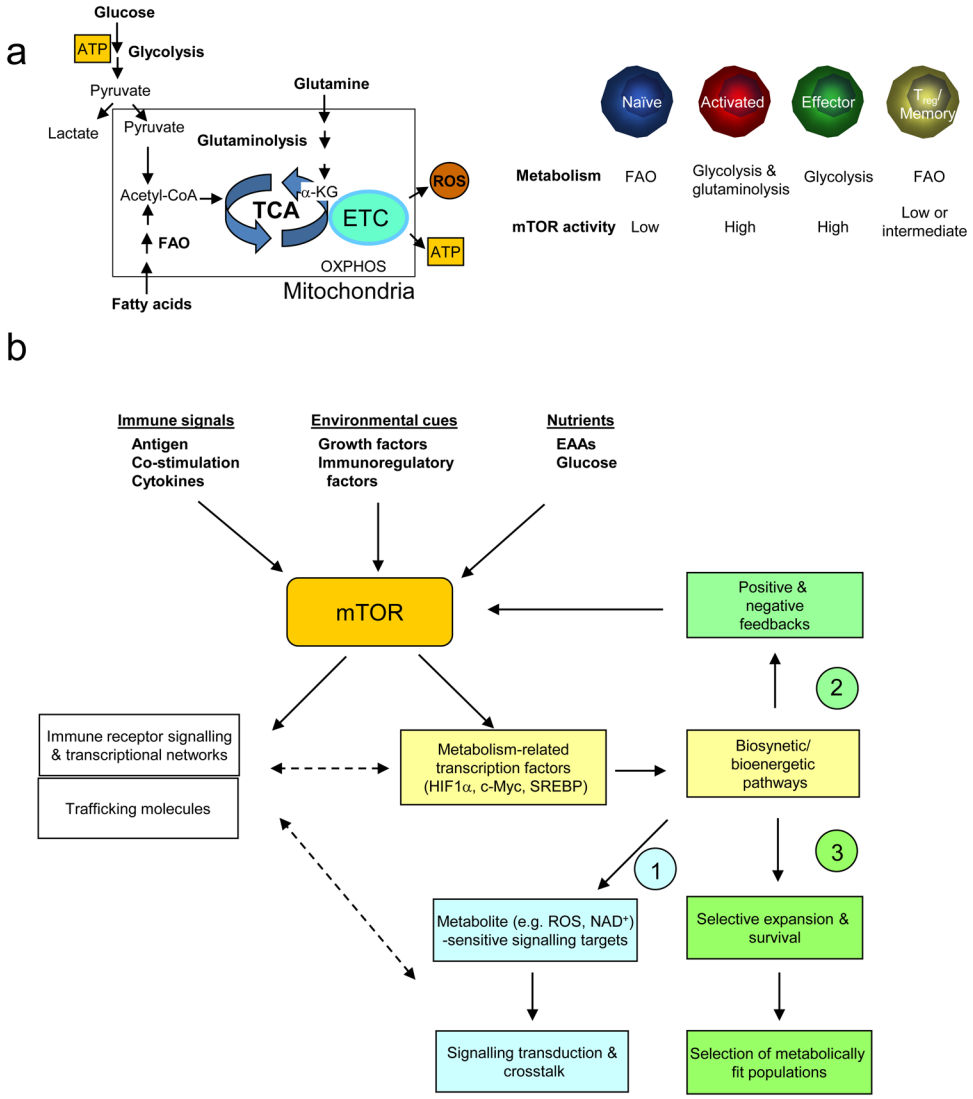



**Figure 3. mTOR in T cell metabolism**

**(a) Metabolic programmes in T cells (left) and mTOR activity in T cell subsets (right).** Only the prominent metabolic programs are presented. FAO, fatty acid oxidation; TCA, tricarboxylic-acid cycle; ETC, electron-transport chain; ROS, reactive oxygen species; OXPHOS, oxidative phosphorylation.

**(b) Proposed mechanisms of mTOR-mediated metabolic pathways in T cell differentiation.** An important upstream signal to activate mTOR is nutrients, in particular essential amino acids (EAAs) whose levels are actively controlled by dendritic cells (DCs). Once activated, mTOR serves as a platform to engage several downstream effector pathways including immune receptor signalling, metabolic programme and migratory activity. mTOR promotes metabolism via activating a gene expression programme consisting of metabolic gene targets of transcription factors hypoxia-inducible factor 1α (HIF1α), c-Myc and SREBP, which in turn impinge upon biosynthetic and bioenergetic pathways. Three potential downstream mechanisms are proposed to explain how these metabolic pathways regulate T cell differentiation, including (1) signal crosstalk; (2) feedback control; (3) selective expansion and survival. Among metabolites with signalling activities, reactive

oxygen species (ROS) oxidize the catalytic cysteine residues of phosphatases to cause their inactivation, whereas $NAD^+$ is required for the activity of sirtuin family deacetylases.

**Table 1**

Genetic models of T cell-specific deletion of mTOR components and negative regulators.

| Target genes | Cre types | Biochemical defects | Thymic phenotypes | Peripheral phenotypes | Refs |
|---|---|---|---|---|---|
| *Mtor* | CD4-Cre | Abrogated mTORC1 and mTORC2 activities | None | Diminished $T_H1$, $T_H2$ and $T_H17$ and spontaneous induced $T_{Reg}$ differentiation | 6, 30 |
| *Rheb* | CD4-Cre | Abrogated mTORC1 activity | None | Diminished $T_H1$ and $T_H17$ and increased $T_H2$ differentiation | 30 |
| *Rictor* | CD4-Cre; dLck-iCre | Abrogated mTORC2 activity | None | Diminished $T_H2$ (both lines) and $T_H1$ (in dLck-iCre) differentiation | 30, 77 |
| *Pten* | CD4-Cre; Lck-Cre | Increased AKT and mTOR activities | Lymphoma; minor defects in development | Autoimmune disease | 44–47, 49–51 |
| *Tsc1* | CD4-Cre; Lck-Cre | Increased mTORC1 and decreased mTORC2 activities | None (CD4-Cre) or minor reduction of cell number (Lck-Cre) | Loss of T cell quiescence and survival; diminished antigen-specific response | 7–9 |
| *Lkb1* | CD4-Cre; Lck-Cre | Diminished AMPK and increased mTORC1 activities | Reduced survival; defective α-selection and positive selection | Reduced survival and TCR-induced proliferation; increased metabolism and cytokine production | 57–60 |

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