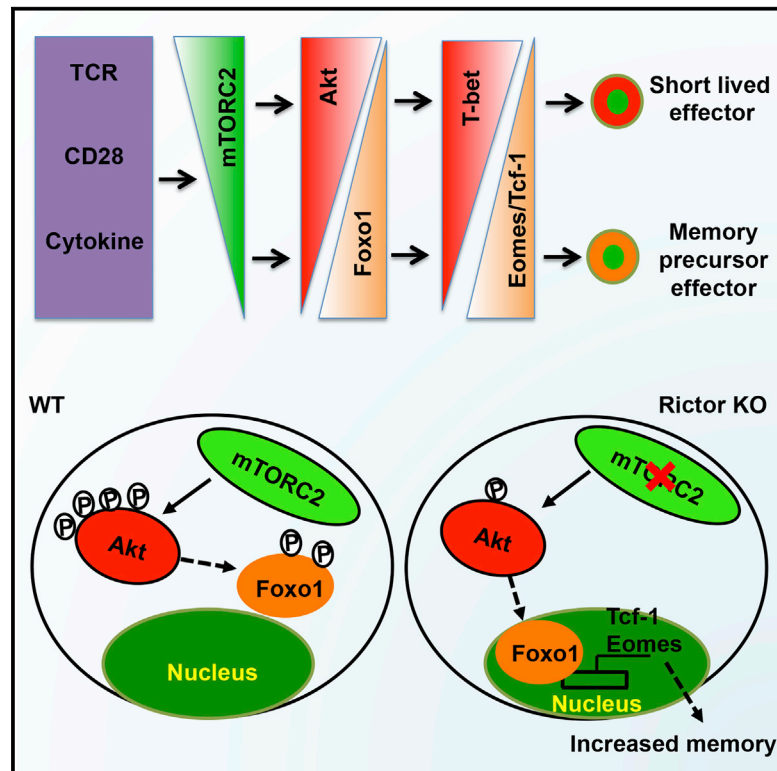# Mammalian Target of Rapamycin Complex 2 Controls CD8 T Cell Memory Differentiation in a Foxo1-Dependent Manner

Graphical Abstract

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In Brief

Zhang et al. demonstrate that mTORC2 deficiency favors CD8 T cell memory differentiation during the primary antigen-specific T cell response to *Listeria* infection. The effects result from higher Foxo1 transcriptional activity without dampening effector functions. They also show enhanced recall responses by mTORC2-deficient memory CD8 T cells.

Highlights

- mTORC2 deficiency promotes MPEC generation and reduces SLECs during *Listeria* infection

- Primed mTORC2-deficient T cells have effector functions and produce higher IL-2 levels

- mTORC2-deficient memory CD8 T cells mount more potent recall responses

- mTORC2 deficiency promotes CD8 memory generation via nuclear accumulation of Foxo1

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Mammalian Target of Rapamycin Complex 2 Controls CD8 T Cell Memory Differentiation in a Foxo1-Dependent Manner

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SUMMARY

Upon infection, antigen-specific naive CD8 T cells are activated and differentiate into short-lived effector cells (SLECs) and memory precursor cells (MPECs). The underlying signaling pathways remain largely unresolved. We show that Rictor, the core component of mammalian target of rapamycin complex 2 (mTORC2), regulates SLEC and MPEC commitment. Rictor deficiency favors memory formation and increases IL-2 secretion capacity without dampening effector functions. Moreover, mTORC2-deficient memory T cells mount more potent recall responses. Enhanced memory formation in the absence of mTORC2 was associated with Eomes and Tcf-1 upregulation, repression of T-bet, enhanced mitochondrial spare respiratory capacity, and fatty acid oxidation. This transcriptional and metabolic reprogramming is mainly driven by nuclear stabilization of Foxo1. Silencing of Foxo1 reversed the increased MPEC differentiation and IL-2 production and led to an impaired recall response of Rictor KO memory T cells. Therefore, mTORC2 is a critical regulator of CD8 T cell differentiation and may be an important target for immunotherapy interventions.

INTRODUCTION

CD8 T cells play a crucial role in immunity to infection and cancer (Chang et al., 2014; Dudda et al., 2013; Kaech and Cui, 2012). Following an acute bacterial or viral infection, naive CD8 T cells are instructed by antigens, co-stimulatory molecules, and cytokines to undergo clonal expansion and functional differentiation into effector or memory T cells. Killer cell lectin-like receptor G1 (KLRG1) and interleukin 7 receptor α (IL-7Rα, CD127) surface markers discriminate short-lived effector cells (SLECs) from memory precursor effector cells (MPECs) (Chang et al., 2014; Kaech and Cui, 2012; Kaech et al., 2003; Sallusto et al., 1999; Wherry et al., 2003). The decision process is affected by inflammation, which triggers distinct transcriptional programs in primed CD8 T cells (Joshi et al., 2007). Multiple transcription factors have been identified to control CD8 effector or memory differentiation (Böttcher and Knolle, 2015; Chang et al., 2014; Kaech and Cui, 2012). The T box transcription factors T-bet and Eomesodermin (Eomes) represent two key transcription factors in orchestrating the SLEC or MPEC fate decision (Chang et al., 2014; Joshi et al., 2007; Kaech and Cui, 2012). In addition, B lymphocyte-induced maturation protein 1 (Blimp-1) and B cell lymphoma 6 (Bcl-6) are reciprocal regulators of CD8 effector and memory differentiation. Blimp-1 enhances the formation of terminally differentiated effectors by directly binding to Bcl-6 to repress its expression (Ji et al., 2011; Kallies et al., 2009; Rutishauser et al., 2009). Moreover, Id2- or Id3-deficient mice fail to generate short-lived effector or long-lived memory cells, respectively (Ji et al., 2011; Yang et al., 2011). However, the T cell intrinsic signaling pathways controlling cell fate decision between SLECs and MPECs remain poorly understood.

Mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase that integrates multiple environmental cues to regulate cell growth, proliferation, and metabolism (Betz and Hall, 2013; Shimobayashi and Hall, 2014). In mammalian cells, mTOR can form functionally distinct mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) multiprotein complexes. Raptor and Rictor are the core adaptor components of mTORC1 and mTORC2, respectively (Betz and Hall, 2013), whose deletion leads to loss of mTORC1 or mTORC2 integrity and activity (Bentzinger et al., 2008). mTORC1 has been shown to regulate both innate and adaptive immunity (Chen et al., 2013; Chi, 2012; Delgoffe et al., 2009; Powell et al., 2012; Waickman and Powell, 2012; Zhu et al., 2014). In T cells, mTORC1 integrates the signals from the T cell receptor
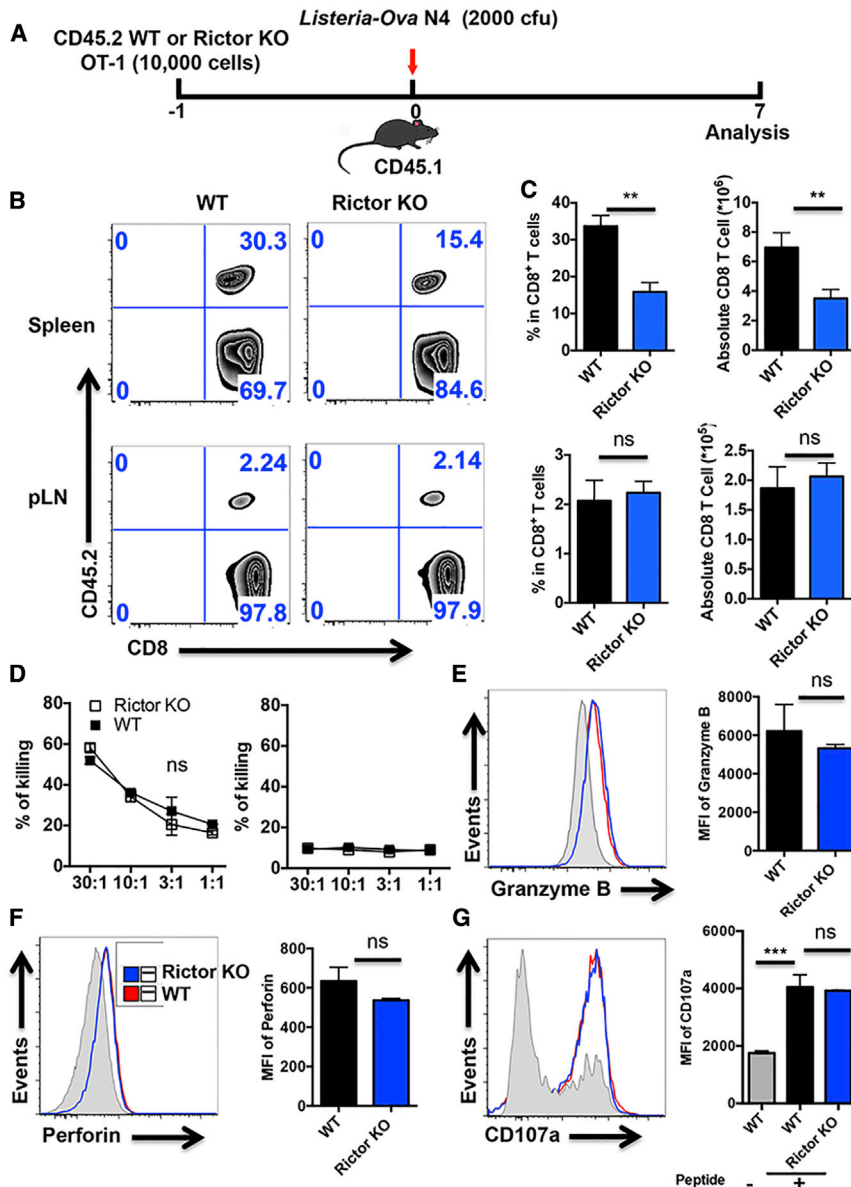
Figure 1. Rictor-Deficient CD8 T Cells Mount Effective Effector Responses to Infection

(A) Schematic of LM-OVA infection. 10, 000 WT or Rictor KO naive OT-1 cells were transferred into naive CD45.1 recipients, followed by injection of 2,000 CFUs of LM-OVA N4 or T4 i.v.

(B) Flow cytometry analysis of CD8 and CD45.2 cells in the spleen and pLNs.

(C) The percentages and absolute numbers of transferred WT or Rictor KO OT-1 cells harvested in the spleen (top) and pLNs (bottom) were quantified after N4 infection.

(D) In vitro killing assay with Cr51 release or sorted WT or Rictor KO effector OT-1 cells on day 7 after infection were incubated with SIINFEKL peptide-pulsed (left) or -non-pulsed (right) EL-4 target cells at different ratios.

(E–G) Representative overlay histograms and the mean fluorescence intensity (MFI) of granzyme B (E), perforin (F), or CD107a (G) expression on transferred WT or Rictor KO OT-1 cells on day 7 after LM-OVA N4 infection. In (E) and (F), the shaded gray histograms represent background levels in gated CD4 T cells. For CD107a, the shaded gray histogram represents WT OT-1 cells without SIINFEKL peptide stimulation.

Data are representative of three or four independent experiments and are presented as the mean ± SEM (4–6 mice/group). **$p < 0.01$; ***$p < 0.001$; ns, not significant. See also Figure S1.

goffe et al., 2011; Heikamp et al., 2014). However, the role mTORC2 may have in shaping CD8 T cell effector and memory generation remains unknown.

RESULTS

Rictor-Deficient CD8 T Cells Mount Effective Effector Responses

To investigate the role of mTORC2 in shaping CD8 effector and memory differentiation, we crossed Rictor knockout (KO) mice onto an OT-1 background and tested how Rictor deficiency would affect the CD8 response to *Listeria monocytogenes* ovalbumin (LM-OVA) infection. Using this system, Zehn et al. (2009) showed that the strength of TCR-ligand interaction critically affects CD8 T cell expansion, contraction, and migration. Therefore, we transferred equal numbers of WT (Rictor^flox/flox: CD4-Cre^−) or Rictor KO (Rictor^flox/flox: CD4-Cre^+) OT-1 cells separately into naive hosts that were then infected with LM recombinants encoding the natural ligand SIINFEKL (N4) or one altered peptide ligand, SIITFEKL (T4), with about 100-fold lower functional avidity for the OT-1 TCR (Zehn et al., 2009; Figure 1A). A similar engraftment efficiency of transferred WT and Rictor KO OT-1 cells was confirmed 1 day later, before LM-OVA infection (data not shown). On day 7 after infection, Rictor KO OT-1 cells showed a 2-fold reduced frequency and absolute number of

(TCR), co-stimulation, cytokines, and nutrients to regulate cell proliferation and differentiation (Zhang et al., 2012). Indeed, mTORC1 is required for Th1, Th2, Th17, and invariant natural killer T (iNKT) cell differentiation (Delgoffe et al., 2009, 2011; Shin et al., 2014; Yang et al., 2013; Zhang et al., 2014). Interestingly, pharmacological inhibition of mTORC1 by rapamycin in acutely *Lymphocytic choriomeningitis virus* (LCMV)-infected mice leads to increased magnitude and qualities of memory CD8 T cell differentiation (Araki et al., 2009; Pearce et al., 2009). Consistently, deletion of TSC1 or TSC2, the upstream inhibitor of mTORC1, drives stronger effector responses but impairs memory formation (Krishna et al., 2014; Shrestha et al., 2014). Recently, the Rictor containing mTORC2 or its downstream target serum- and glucocorticoid-regulated kinase (SGK1) have been shown to promote Th2 differentiation (Del-
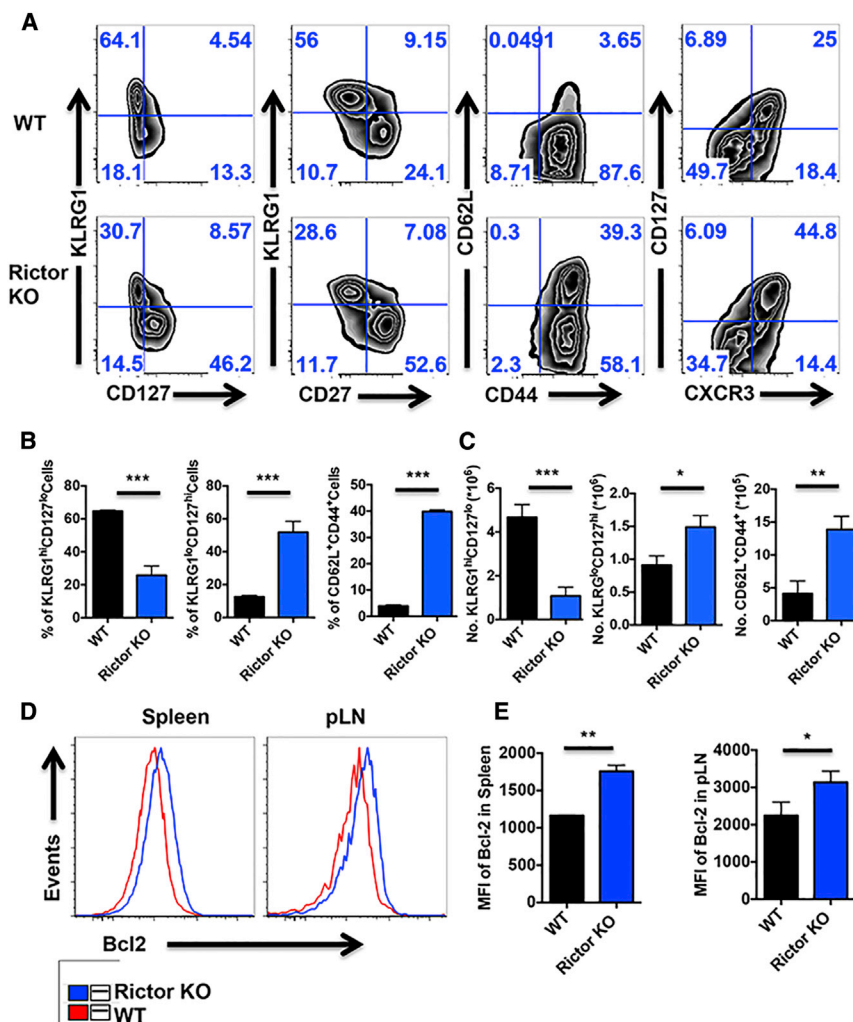
Figure 2. Rictor Deficiency Leads to Enhanced MPEC Generation while Reducing SLECs.

(A) Representative FACS plots showing KLRG1, CD127, CD27, CD62L, CD44, and CXCR3 staining patterns on gated transferred OT-1 cells in the spleen on day 7 after LM-OVA N4 infection.

(B and C) The frequencies (B) and absolute numbers (C) of KLRG1^{hi} CD127^{lo}, KLRG1^{lo} CD127^{hi}, and CD62L$^+$CD44$^+$ on gated transferred OT-1 cells in the spleen are summarized.

(D) Intracellular Bcl-2 expression was determined by flow cytometry.

(E) The MFI of Bcl-2 was determined in the spleen (left) and pLN (right).

Data are representative of three or four independent experiments and are presented as the mean ± SEM (4–6 mice/ group). *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S2.

Rictor KO effector cells could kill target EL-4 cells pulsed with SIINFEKL peptide as efficiently as WT effectors (Figure 1D). Granzyme B and perforin are the key effector molecules responsible for direct cytotoxicity of CD8 T cells. In line with comparable killing ability in vitro, both WT and Rictor KO effector cells expressed similar high levels of granzyme B and perforin (Figures 1E and 1F). Furthermore, CD107a expression, a degranulation marker correlated with cytolytic capacity, was strongly upregulated at the surface membrane of Rictor KO effector cells upon in vitro peptide restimulation (Figure 1G). Together, our data suggest that acquisition of CD8 T cell effector function is independent of mTORC2.

Rictor Deficiency Leads to Enhanced MPEC Generation while Reducing SLECs

Upon infection, antigen-primed CD8 T cells undergo differentiation into SLECs and MPECs. SLECs, defined as KLRG1^{hi} CD127^{lo}, undergo massive apoptosis during the contraction phase after the infection clears, whereas KLRG1^{lo} CD127^{hi} MPECs show increased potential to differentiate further into long-lived memory lineage cells. To examine whether mTORC2 regulates SLEC and MPEC commitment, we determined the expression profiles of KLRG1, CD127, and CD27 on WT or Rictor KO effector OT-1 cells at the peak of the response to primary infection. We found a significant reduction of KLRG1^{hi} CD127^{lo} SLECs in the absence of Rictor (Figures 2A and 2B). Conversely, the levels of KLRG1^{lo} CD127^{hi} MPECs were 2- to 3-fold higher in Rictor KO than in WT responding cells (Figures 2A–2C). Consistently, we also found increased percentages of the KLRG1^{lo} CD27$^+$ population in the absence of Rictor (Figure 2A). Strikingly, we observed a marked increase (more than 10-fold) in the

effector cells compared with the wild-type (WT) in response to either N4 or T4 stimulation in the spleen, liver, lung, and bone marrow (Figures 1B, 1C, and S1A−S1C; data not shown). However, the levels of transferred CD8 T cells in the peripheral lymph node (pLN) were comparable, suggesting that T cell trafficking might also be regulated by mTORC2 (Figures 1B and 1C). To determine whether the reduced CD8 T cell response in the absence of Rictor was due to decreased proliferation or increased apoptosis, we first measured Ki67 expression on day 5 after infection. As expected, N4 infection triggered higher Ki67 expression than T4, confirming the higher expansion capacity upon strong ligand stimulation (Figure S1D). Consistent with the reduced CD8 T cell levels, Ki67 expression in Rictor KO OT-1 cells was reduced compared with WT OT-1 cells after N4 infection (Figure S1D). In contrast, Rictor deficiency did not detectably affect the apoptosis rate of effector CD8 T cells (Figure S1E). These data suggest that mTORC2 is required for CD8 sustained proliferation and accumulation.

Next we examined whether Rictor-deficient CD8 T cells were capable of killing antigenic target cells. We found that
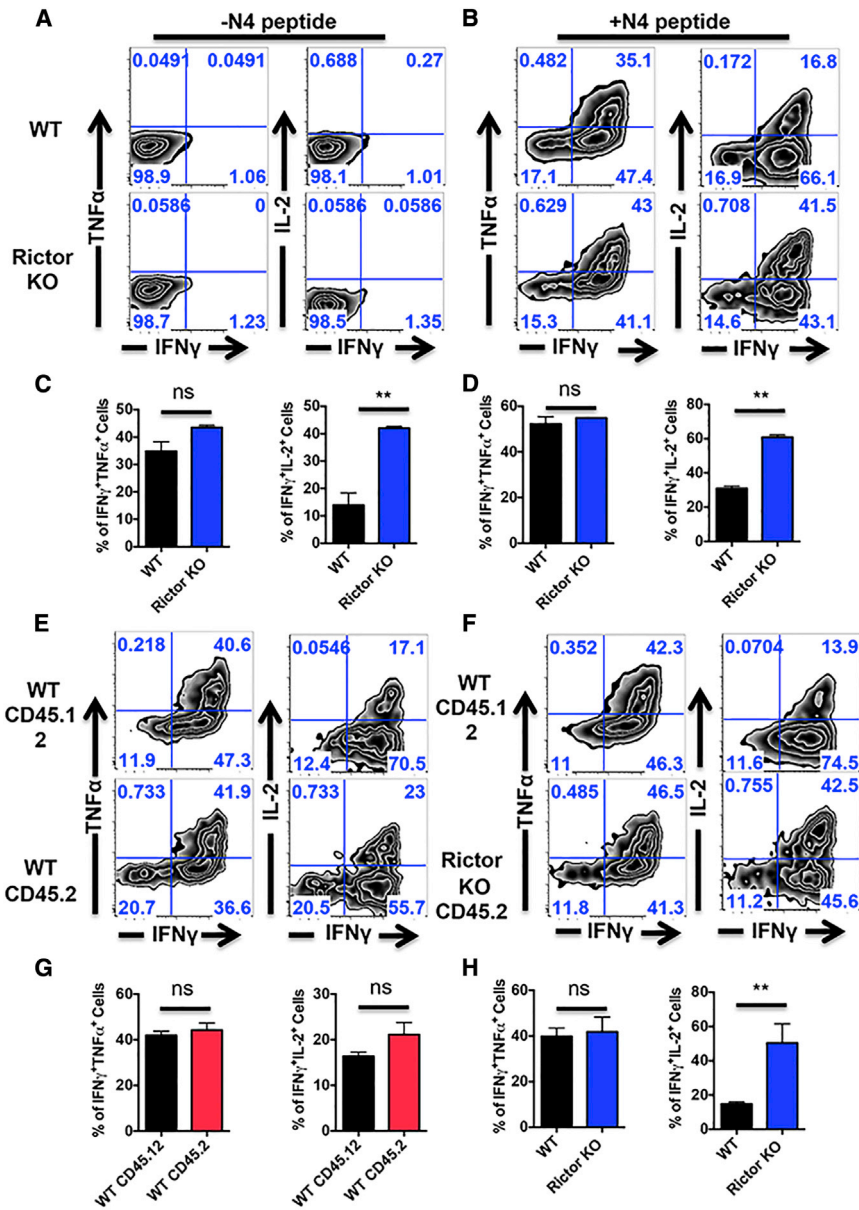
Figure 3. Rictor Deficiency Promotes Cell-Autonomous IL-2 Production in CD8 T Cells.

(A and B) On day 7 after LM-OVA infection, splenocytes from CD45.1 recipients transferred with WT or Rictor KO OT-1 cells were isolated and re-stimulated without (A) or with (B) the SIINFEKL peptide for IFNγ, TNF-α and IL-2.

(C and D) The frequencies of IFNγ⁺TNF-α⁺ and IFNγ⁺IL-2⁺ OT-1 cells in the spleen (C) and pLNs (D).

(E–H) WT CD45.1/2⁺ naive OT-1 cells were mixed at a 1:1 ratio with naive WT CD45.2⁺ OT-1 cells (E and G) or Rictor KO CD45.2⁺ OT-1 cells (F and H) and transferred into CD45.1⁺ recipients, followed by LM-OVA N4 infection 1 day later. On day 7 after LM-OVA infection, splenocytes were re-stimulated with the SIINFEKL peptide for IFNγ, TNF-α, and IL-2.

Data are representative of three or four independent experiments and are presented as the mean ± SEM (4–5 mice/group). **p < 0.01. See also Figure S3.

To exclude any effects from the inflammatory microenvironments in different hosts, we adoptively co-transferred mixtures of WT CD45.1/2⁺ OT-1 cells with Rictor KO CD45.2⁺ OT-1 cells at a 1:1 ratio into the same host. Even in this setting, higher MPEC and reduced SLEC frequencies were observed in both the spleen and pLNs (Figures S2B and S2C; data not shown). The increased proportions of the Rictor-deficient KLRG1^lo CD127^hi or CD62L⁺ CD44⁺ populations were even more striking in the host pLN (data not shown). Taken together, these findings support a CD8 T cell-intrinsic role of mTORC2 in promoting MPEC while inhibiting SLEC differentiation.

Rictor Deficiency Leads to Enhanced Cell-Autonomous IL-2 Production in CD8 T Cells

At the peak of the primary T cell response to acute infection, only a small fraction of effector CD8 T cells is able to produce IL-2 (Joshi et al., 2007; Sarkar et al., 2008). On day 7 after LM-OVA N4 infection, about 15% of WT effector CD8 T cells secreted IL-2 (Figures 3A–3D). Most intriguingly, we found a remarkably increased frequency of IL-2-producing, Rictor-deficient CD8 T cells (Figures 3B–3D). In contrast, they produced similar amounts of interferon γ (IFNγ) and tumor necrosis factor α (TNF-α) in response to Ova-N4 peptide stimulation as WT CD8 T cells (Figures 3C and 3D). Moreover, we found that IL-2 production was also increased significantly in Rictor KO effector cells in response to LM-OVA T4 infection (Figure S3). Similarly, we reproduced the finding that Rictor KO effectors from LM-OVA N4 infection produced higher levels of IL-2 even with in vitro OVA-T4 peptide stimulation (Figure S3). Moreover, higher IL-2 production was also observed in Rictor KO effectors from LM-OVA T4 infection in the presence

frequency of Rictor KO OT-1 cells with the CD62L⁺ CD44⁺ central memory phenotype, whereas WT OT-1 cells rarely harbor this phenotype at this early time point after infection (Figures 2A–2C). Additionally, the percentages of CD127⁺ CXCR3⁺ cells were also enhanced in the absence of mTORC2 (Figure 2A). We also found a much higher intensity of CD62L, CD127, and CD122 expression on a per-cell basis in the absence of mTORC2 (Figure 2A; data not shown), indicating the potential for Rictor KO cells to respond better to IL-7 or IL-15 stimulation. The increased expression of CD62L and CCR7 was also confirmed at the transcriptional level (Figure S2A). It has been shown that Bcl-2 expression is higher in MPECs that will differentiate further into a long-lived memory lineage. Indeed, we found that Bcl-2 expression was much higher in Rictor KO CD8 T cells than that of WT counterparts (Figures 2D and 2E).
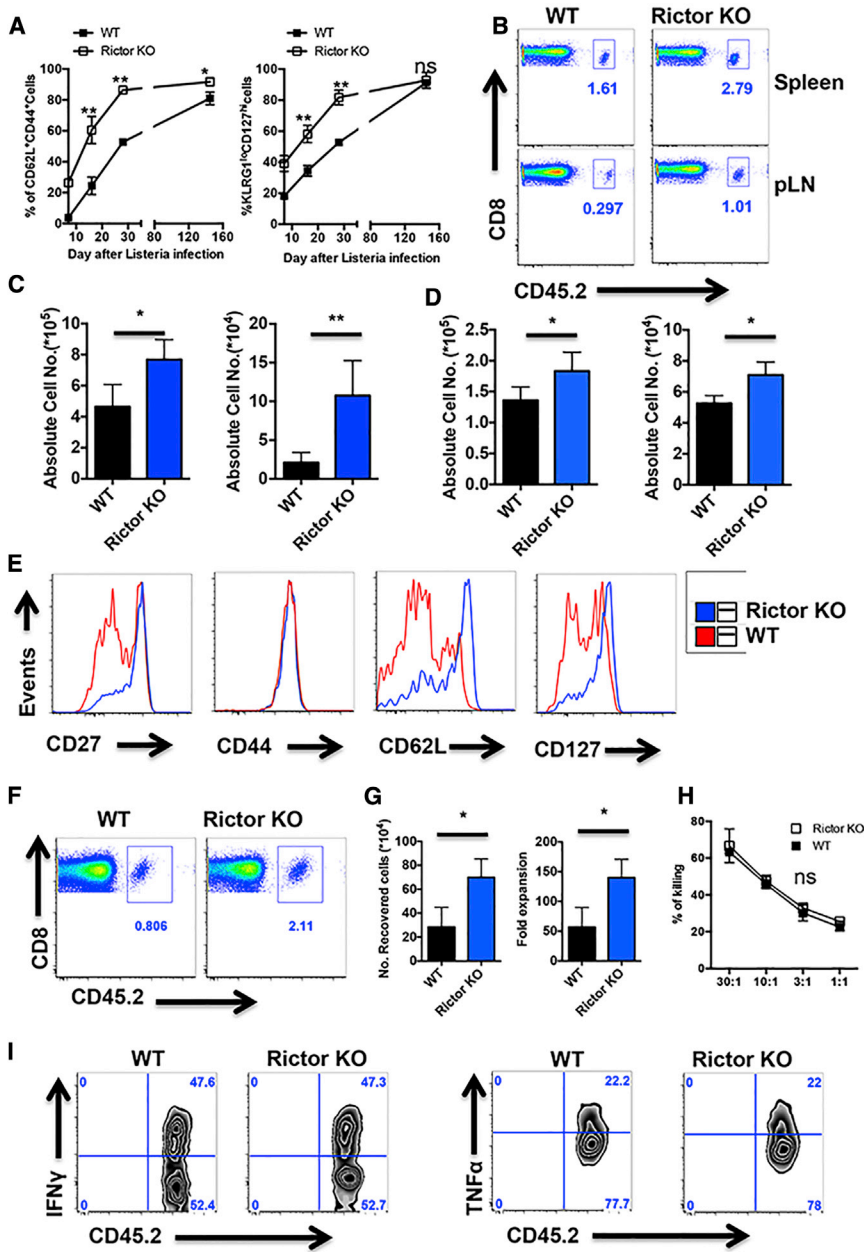
Figure 4. Rictor Deficiency Leads to Enhanced CD8 Memory T Cell Formation and Better Recall Responses.

(A) Kinetic analysis of the frequency of CD62L$^+$CD44$^+$ or KLRG^{lo} CD127^{hi} on transferred WT or Rictor KO CD45.2 OT-1 cells at different time points after N4 infection.

(B) Representative FACS plots showing the CD8 and CD45.2 staining patterns 7 weeks after infection in the spleen and pLNs, gated on CD8 T cells.

(C) The absolute numbers of transferred WT or Rictor KO CD45.2 OT-1 cells in the spleen (left) and pLN (right).

(D) The absolute numbers of transferred WT or Rictor KO CD45.2 OT-1 cells in the liver (left) and lung (right).

(E) Representative FACS plots showing the expression pattern of CD27, CD44, CD62L, and CD127 on transferred WT or Rictor KO OT-1 cells in the spleen.

(F) Representative FACS plots showing CD8 and CD45.2 cells on day 4 after antigen re-exposure in the spleen.

(G) Absolute number or fold expansion of WT or Rictor KO OT-1 cells after re-challenge in the spleen.

(H) In vitro Cr51 release-based killing assay with sorted WT or Rictor KO effector OT-1 cells on day 4 after re-challenge, incubated with SIINFEKL peptide-pulsed EL-4 target cells at different ratios.

(I) On day 4 after re-challenge, splenocytes from CD45.1 recipients were isolated and re-stimulated with the SIINFEKL peptide for intracellular staining of IFNγ and TNF-α.

Data are representative of two or three independent experiments and are presented as the mean ± SEM (4–5 mice/group). *p < 0.05, **p < 0.01. See also Figures S3 and S4.

manner to selectively increase IL-2 production in response to antigen challenge.

Rictor Deficiency Leads to Enhanced Long-Lived CD8 Memory T Cell Differentiation

It has been reported that superior IL-2-producing MPECs differentiate more efficiently into long-lived protective memory T cells capable of self-renewal and rapid recall responses (Joshi et al., 2007; Sarkar et al., 2008). Given the enhanced differentiation into high IL-2-producing MPECs, we investigated whether Rictor deficiency could give rise to increased long-lived memory T cell differentiation. We found that the higher levels of KLRG1^{lo} CD127^{hi} or CD62L$^+$ CD44$^+$ CD8 T cells were maintained stably throughout the observation time of 4 months post-infection (Figure 4A). This was also confirmed in the co-transfer setting (Figures S3B and S3C). Seven weeks after primary infection, we indeed observed higher frequencies and absolute numbers of transferred Rictor KO OT-1 cells than of WT OT-1 cells in both the spleen and pLN, with a more striking increase in pLN (Figures

of OVA-N4 peptide stimulation in vitro (Figure S3), suggesting that higher IL-2 production in Rictor-deficient CD8 T cells was programmed regardless of infection with high- or low-affinity ligand. Enhanced IL-2 production in the absence of functional mTORC2 was confirmed further in co-transfer experiments (Figures 3E–3H). Indeed, although, in the WT CD45.1/2$^+$ 1:1 WT CD45.2$^+$ OT-1 control co-transfer, similar IFNγ, TNF-α, and IL-2 production was apparent upon peptide restimulation in vitro (Figures 3E and 3G), a significant increase in IL-2 production in the absence of Rictor was found in the WT CD45.1/2$^+$ 1:1 Rictor KO CD45.2$^+$ OT-1 transfer settings, whereas IFNγ and TNF-α secretion remained the same (Figures 3F and 3H). These results indicate that Rictor deficiency works in a CD8 T cell-intrinsic
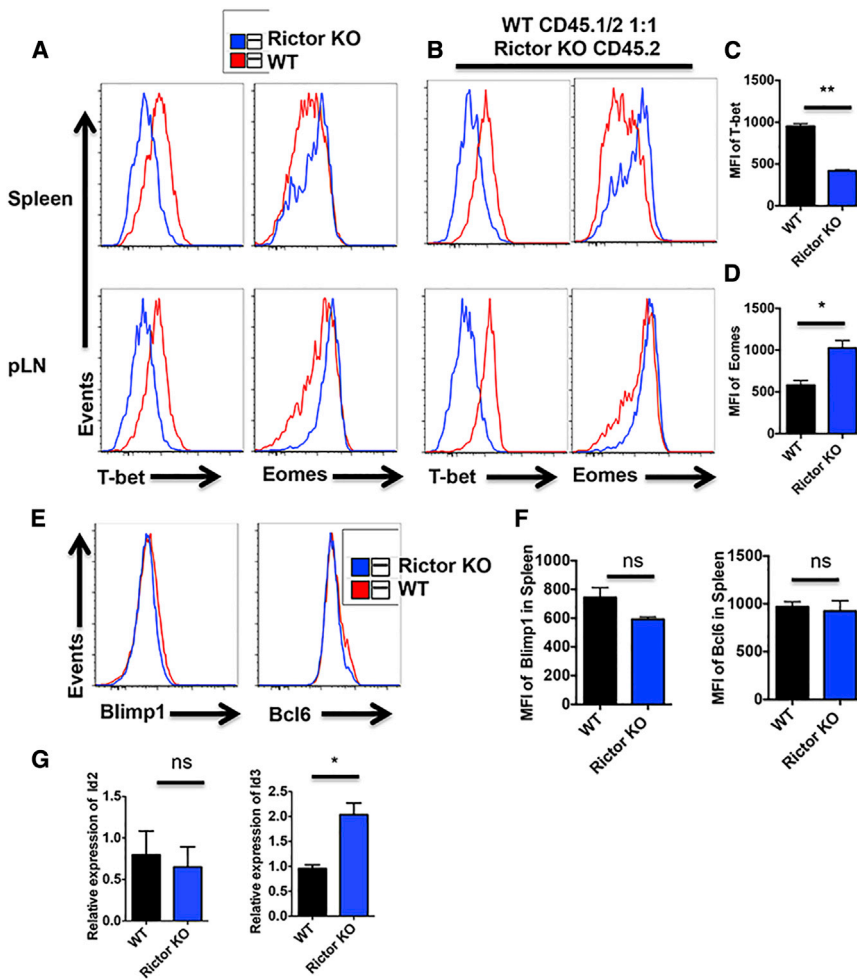
Figure 5. Rictor Deficiency Alters the Balance of T-bet and Eomes Expression.

(A) Representative overlay histograms showing T-bet and Eomes expression on day 7 after infection.

(B) Representative overlay histograms showing T-bet and Eomes expression in the mix on transfer day 7 after infection.

(C and D) As shown in (A), the MFIs of T-bet (C) and Eomes (D) in the spleen are summarized.

(E) Representative overlay histograms showing Blimp1 and Bcl-6 expression on day 7 after infection in the spleen.

(F) The MFI of Blimp1 and Bcl-6 is summarized.

(G) On day 7 after infection, transferred OT-1 effector cells were sorted for real-time PCR analysis of Id2 and Id3 expression.

Data are representative of three or four independent experiments and are presented as the mean ± SEM (4–6 mice/group). *p < 0.05, **p < 0.01. See also Figure S5.

compared with WT cell transfer (Figures 4F and 4G), directly implying that Rictor deficiency increases potently functional memory CD8 T cells. Interestingly, recalled Rictor KO OT-1 cells retained higher CD62L expression. In addition, they upregulated CXCR3 expression better, which is important for homing to inflammation sites (Figure S4A). Furthermore, we did not observe any difference in the killing capacity between WT and Rictor KO secondary effector cells (Figure 4H). We also assessed cytokine production by memory T cells after antigen re-exposure. Rictor KO secondary effectors indeed produced similar amounts of IFNγ and TNF-α upon restimulation (Figures 4I, S4B, and S4C). Together, these results show that Rictor-deficient memory T cells mount more potent recall responses while preserving WT-like immediate effector function.

Enhanced CD8 T Cell Memory Formation in the Absence of Rictor Is Associated with Transcriptional and Metabolic Reprogramming

The T box transcription factors T-bet and Eomes control the cell fate decision between SLECs and MPECs (Intlekofer et al., 2005; Rao et al., 2012). A higher T-bet/Eomes ratio drives terminal effector differentiation, whereas a higher Eomes/T-bet ratio favors memory formation. We therefore examined the expression pattern of T-bet and Eomes in WT and Rictor KO OT-1 cells on day 7 after LM-OVA infection. Unexpectedly, given the similar level of effector cytokine production, we observed a significant decrease in T-bet expression and concomitantly increased Eomes expression in Rictor-deficient cells (Figures 5A–5D). Furthermore, this higher Eomes, lower T-bet expression pattern in Rictor KO cells was maintained stably even at the contraction (day 14 after infection; Figures S5A and S5B) or memory phases

4B and 4C; data not shown). Meanwhile, the absolute numbers of memory CD8 T cells in non-lymphoid tissues like liver and lungs were also increased slightly but significantly (Figure 4D). At this time point, we also confirmed a higher expression level of CD27, CD62L, and CD127 in the absence of Rictor, whereas the CD44 expression remained similar (Figure 4E). This increased CD62L and CD127 expression was still observed 7 months after primary infection (data not shown). Together, these data support increased memory CD8 T cell generation in Rictor-deficient T cells.

Rictor KO Memory T Cells Mediate More Potent Recall Responses

A unique feature of memory T cells is their ability to mediate an accelerated recall response with higher magnitude upon antigen re-exposure. To determine the role of mTORC2 in this process, we transferred similar numbers of WT or Rictor KO memory CD8 T cells sorted from mice 7 weeks after the primary challenge into naive recipients. The secondary hosts were challenged 1 day later with LM-OVA. On day 4 after infection, we found significantly increased frequencies and absolute numbers of Rictor KO OT-1 cells in the spleen and liver (data not shown)
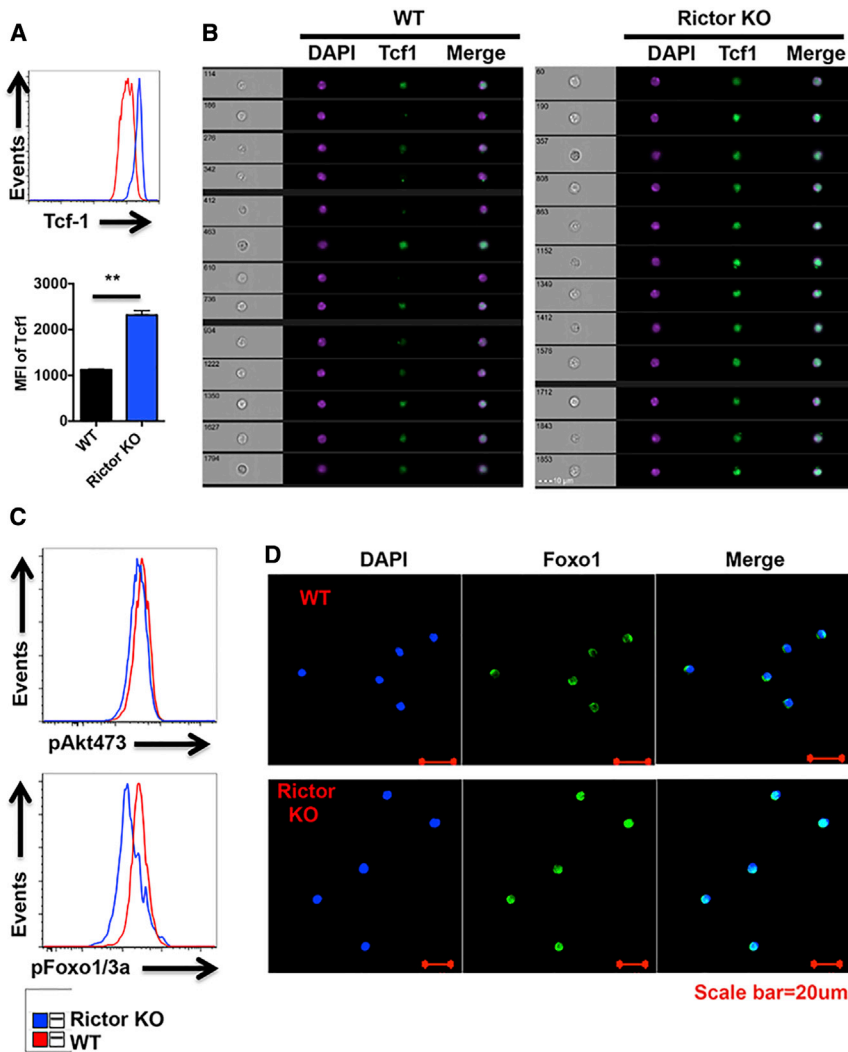
Figure 6. Rictor Deficiency Promotes Tcf-1 Expression and Foxo1 Nuclear Accumulation.

(A) Top: representative overlay histograms showing Tcf-1 expression on day 14 after infection in the spleen. Bottom: the MFI of Tcf-1 expression in the spleen is summarized.

(B) Imagestream analysis of Tcf-1 expression on day 14 after infection. DAPI was used as a counterstain to determine the localization of Tcf-1 in the nucleus.

(C) Representative overlay histograms showing pAkt473 (top) and pFoxo1/3a (bottom) expression 4.5 days after infection in the spleen.

(D) Foxo1 localization was determined with confocal microscopy 4.5 days after LM-OVA N4 infection in the spleen.

Data are representative of two or three independent experiments and are presented as the mean ± SEM. **p < 0.01. See also Figure S5.

(data not shown). Blimp1 drives terminal differentiation into effectors cells, whereas Bcl-6 promotes MPEC differentiation (Ji et al., 2011; Rutishauser et al., 2009). However, we did not observe any detectable change in Blimp1 and Bcl-6 expression with Rictor deficiency (Figures 5E and 5F). Id2 and Id3 have been shown to control CD8 effector and memory differentiation (Ji et al., 2011; Yang et al., 2011). Consistent with increased memory formation, we found a slight but significant increase in Id3 expression at the transcription level, whereas Id2 expression remained comparable (Figure 5G).

Tcf-1, the downstream target of the Wnt signaling pathway, is required for CD8 memory generation and recall response (Jeannet et al., 2010; Zhou et al., 2010). Consistent with increased CD8 memory formation in the absence of Rictor, Tcf-1 expression was enhanced significantly in Rictor KO cells compared with the WT on day 14 after primary infection in all organs assessed (Figures 6A, 6B, S5C, and S5D). Both the Tcf-1 nuclear localization pattern and higher expression in the absence of Rictor could be visualized directly at the single-cell level and quantified by Imagestream flow analysis (Figure 6B).

In terms of metabolic activity, primed CD8 T cells switch from oxidative phosphorylation (OXPHOS) to glycolysis, whereas CD8 memory T cells, unlike effector or naive CD8 T cells, exhibit substantial spare respiratory capacity (SRC) in their mitochondria (van der Windt et al., 2012; Waickman and Powell, 2012). IL-15, a crucial cytokine supporting CD8 memory T cell generation, regulates SRC and oxidative metabolism by promoting carnitine palmitoyl transferase (CPT1α) expression (van der Windt et al., 2013). To understand the connection between CD8 effector or memory differentiation and metabolic changes brought about by dysregulated mTORC2 signaling, we primed WT or Rictor KO naive OT-1 cells with SIINFEKL peptide and IL-2 for 3 days and continued to culture in the presence of IL-2 or IL-15 for another 3-4 days to generate in vitro "effector" or "memory" cells, respectively. First, we confirmed the increased SRC with IL-15-treated WT CD8 T cells compared with IL-2 cultured cells (Figures S6A and S6B). Consistent with enhanced memory differentiation, Rictor-deficient OT-1 cells cultured in IL-15 showed increased SRC compared with the WT. Interestingly, even when cultured in the presence of IL-2, which promotes effector differentiation, Rictor KO cells showed a striking increase in SRC (Figures S6A and S6B). Memory CD8 T cells preferentially use mitochondrial fatty acid oxidation (FAO) for their energy supply (Pearce et al., 2009; van der Windt et al., 2013). By using etomoxir, an inhibitor of CPT1α, we observed a pronounced decrease in oxygen consumption rate (OCR) (an indicator of OXPHOS) in either WT or Rictor KO CD8 T cells cultured with IL-15, suggesting that these memory CD8 T cells highly depend on FAO (Figures S6C and S6D). In contrast, WT CD8 T cells cultured in IL-2 are less dependent on FAO to generate energy (Figures S6C and S6D).

Intriguingly, our results showed that, in contrast to WT CD8 T cells, Rictor KO CD8 T cells differentiated under effector conditions rely heavily on CPT1α-dependent fatty acid metabolism to sustain their OCR, a cardinal metabolic feature of memory T cells. A higher level of FAO was further confirmed by increased CPT1α expression in Rictor KO cells (Figure S6G).

To test whether mitochondria were involved in the metabolic reprogramming by mTORC2 deficiency, we first determined the mitochondrial mass by using MitoTracker staining for in vitro-cultured WT and Rictor KO OT-1 cells. Indeed, Rictor KO OT-1 cells showed increased mitochondrial mass compared with WT OT-1 cells (Figure S6E). In addition, the ratio of mitochondrial DNA versus nuclear DNA was also increased significantly in Rictor KO cells (Figure S6F). Ppargc-1α (PGC1α) plays a key role in mitochondrial biogenesis and function by inducing the expression of nuclear respiration factor 1 (Nrf-1) and mitochondrial transcriptional factor A (Tfam). Consistent with the increased mitochondrial content, we observed an enhanced expression of PGC1α, PGC1β, and Tfam in Rictor KO cells (Figure S6G). Moreover, we found that the expression of several essential components of the mitochondrial respiratory chain complex was upregulated (data not shown). Together, these observations support the fact that Rictor deficiency might promote memory T cell metabolic features by increasing mitochondrial function.

Increased Nuclear Foxo1 Accumulation Mediates Enhanced Memory Formation in Rictor-Deficient CD8 T Cells

Foxo1 promotes CD8 memory generation by directly controlling Eomes and Tcf-1 expression (Hess Michelini et al., 2013; Kim et al., 2013; Rao et al., 2012; Tejera et al., 2013). Loss of Foxo1 does not affect CD8 effector responses but impairs the recall response. Foxo1 is mainly phosphorylated by Akt or other SGC family kinases. Phosphorylation of Foxo1 leads to nuclear exit and cytoplasmic localization, resulting in loss of transcriptional activity. Given the enhanced Eomes and Tcf-1 expression in Rictor KO T cells, we hypothesized higher Foxo1 transcriptional activity through its nuclear accumulation in the absence of mTORC2 signaling. Therefore, we determined the levels of pAkt473 and pFoxo1 expression. Phosphorylation of both Foxo1T24/Foxo3aT32, Foxo1S256 (data not shown), and AktS473 sites were clearly reduced in Rictor KO T cells on day 5 after LM-OVA infection, as determined by fluorescence-activated cell sorting (FACS) and western blotting (Figure 6C; data not shown). In line with this finding, Foxo1 was mostly localized to the nuclei of Rictor KO cells, whereas it was mainly cytoplasmic in WT T cells (Figure 6D).

To further substantiate the role of Foxo1 in transcriptional reprogramming in Rictor-deficient T cells, we knocked down Foxo1 with short hairpin RNA (shRNA) and asked whether reduction of Foxo1 could reverse the increased memory differentiation phenotype in the Rictor KO setting. We first confirmed the efficiency and specificity of the Foxo1 shRNA (Figure 7A). We then transferred either scramble- or Foxo1 shRNA-transduced Rictor KO OT-1 cells into CD45.1 naive hosts, followed by LM-OVA infection. Interestingly, Foxo1

shRNA-transduced GFP+ OT-1 cells showed reduced MPEC differentiation compared with the non-transduced GFP− population (Figure 7B). In contrast, the scramble-transduced population did show MPEC differentiation similar to the untransduced internal controls (data not shown). Moreover, compared with scramble-transduced cells, Foxo1 shRNA-transduced OT-1 cells indeed produced less IL-2 (Figure 7C). Together, these results demonstrate that Foxo1 mediates increased MPEC differentiation and IL-2 production in Rictor-deficient CD8 T cells (Figure 7H). Consistently, Foxo1 knockdown (KD) reduced the expression of CD62L and CD127 (data not shown). This was also reflected by the upregulation of T-bet but decreased Eomes and Tcf-1 expression in Foxo1 KD cells (Figures 7D and 7E). More importantly, silencing of Foxo1 led to an impaired recall response of Rictor KO memory T cells. Untransduced Rictor memory KO CD8 T cells (GFP−) could mediate robust recall response upon rechallenge. However, memory Rictor KO T cells transduced with Foxo1 shRNA showed a comparatively largely impaired re-expansion potential upon antigen rechallenge (Figures 7F and 7G).

To investigate whether Foxo1 was involved in the metabolic regulation of CD8 memory formation, we transduced WT OT-1 cells with the Foxo1 AAA mutant, which is constitutively active. Indeed, we observed the upregulation of CPT1α, a key enzyme for the β-oxidation of long-chain fatty acids (data not shown). Importantly, these Foxo1 AAA-transduced WT OT-1 cells show enhanced SRC-like Rictor KO T cells (Figures S7A and S7B). Concomitantly, Foxo1 AAA-transduced WT OT-1 cells show higher FAO because administration of etomoxir largely reduced the oxygen consumption rate compared with untransduced cells (GFP−). Interestingly, these Foxo1 AAA-transduced cells are even more dependent on FAO than the Rictor KO cells (Figures S7C and S7D).

DISCUSSION

In this study, we identify the mTORC2-Akt-Foxo1 signaling axis as a crucial regulator of CD8 T cell effector and memory differentiation via specific transcriptional reprogramming.

We show enhanced memory differentiation and better recall response upon re-challenge by CD8 T cells lacking Rictor. We further demonstrate that mTORC2 controls CD8 memory T cell differentiation in a Foxo1-dependent manner. Knocking down Foxo1 reverses the increased MPEC differentiation and IL-2 production in Rictor KO cells. Foxo1 regulates multiple facets of T cell biology by controlling proliferation, differentiation, and trafficking. In CD8 T cells, Foxo1 is dispensable for a primary response upon infection. However, memory formation and recall response were largely impaired in the absence of Foxo1. In the absence of mTORC2, increased Foxo1 retention in the nucleus of CD8 T cells drives enhanced Eomes and Tcf-1 expression, which promotes CD8 memory differentiation (Figure 7H). Indeed, Foxo1 directly induced expression of both Eomes and Tcf-1, as shown by chromatin immunoprecipitation sequencing experiments (Hess Michelini et al., 2013; Kim et al., 2013; Rao et al., 2012; Tejera et al., 2013). Moreover, it is known that Foxo1 acts as a repressor of T-bet expression in CD8 T cells (Rao
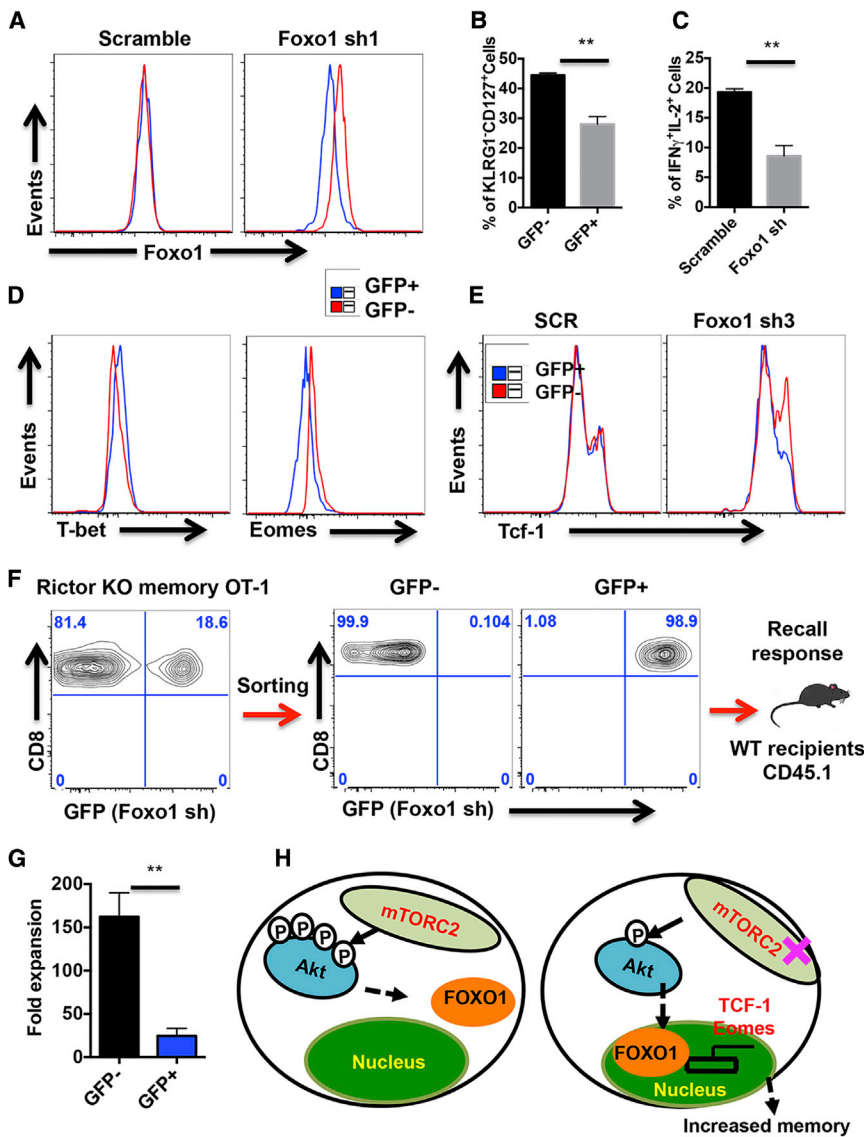
Figure 7. Rictor Deficiency Promotes CD8 Memory Differentiation in a Foxo1-Dependent Manner

Rictor KO CD45.2 OT-1 cells were transduced with either scramble or Foxo1-GFP shRNA and then transferred into naive CD45.1 recipients, followed by infection.

(A) Representative overlay histograms showing decreased Foxo1 expression with Foxo1 shRNA (right) but not with scramble-transduced settings (left).

(B) The frequencies of cells displaying the KLRG1^{lo}CD127^{hi} phenotype in Rictor KO OT-1 cells transduced with either scrambled or Foxo1 shRNA are summarized.

(C) The frequencies of IFNγ$^+$IL-2$^+$-producing cells upon scramble or Foxo1 shRNA transduction on day 7 after infection.

(D) Representative overlay histograms showing T-bet and Eomes expression gated on Foxo1 shRNA-transduced (blue) or non-transduced (red) Rictor KO OT-1 cells on day 7 after infection.

(E) Representative overlay histograms showing Tcf-1 expression on scramble (SCR)- or Foxo1 shRNA-transduced cells on day 7 after infection.

(F) 6 weeks after infection, Foxo1 shRNA-transduced or non-transduced memory Rictor KO OT-1 cells were sorted and transferred into a naive host, followed by re-challenge.

(G) On day 5 after re-challenge, the fold expansion was calculated on Foxo1 shRNA-transduced or non-transduced memory cells.

(H) Schematic of mTORC2 in regulating CD8 T cell memory formation upon infection. Absence of Rictor leads to decreased Akt473 phosphorylation upon stimulation and, therefore, phosphorylation of Foxo1 is decreased. Thus, Foxo1 nuclear retention drives Tcf1 and Eomes expression, which promotes CD8 T cell memory differentiation. Data are representative of two or three independent experiments and are presented as the mean ± SEM. **p < 0.01. See also Figures S6 and S7.

et al., 2012). Overexpression of Foxo1 inhibits T-bet expression, whereas Foxo1-deficient CD8 T cells show higher expression of T-bet (Rao et al., 2012). In line with these observations, we show much lower expression of T-bet in the absence of functional mTORC2. Silencing of Foxo1 restores T-bet expression while reducing Eomes and Tcf1 expression. Interestingly, upon rechallenge, secondary Rictor KO effector cells also show higher Eomes and lower T-bet expression (data not shown), indicating a higher possibility of enhanced secondary memory formation.

The involvement of mTORC1 in regulating CD8 memory differentiation has been suggested by administration of rapamycin and metformin (Araki et al., 2009; Pearce et al., 2009). In the case of rapamycin administration during acute LCMV infection, the enhanced memory differentiation could be phenocopied by silencing of Raptor in CD8 T cells (Araki et al., 2009). In marked contrast, Raptor KO CD8 T cells largely failed to generate effectors and also failed to generate a memory response (L.Z. unpub-

lished data). This discrepancy suggests that fine-tuning of mTORC1, but not its ablation, is required for the enhancement of memory T cell formation. This remains to be addressed experimentally. Alternatively, however, out data raise the possibility that rapamycin may, in fact, exert its positive effect on memory T cell formation via the inhibition of mTORC2 signaling in CD8 T cells in addition to inhibiting mTORC1. Such cell type variation in the ability of rapamycin to inhibit mTORC2 has been shown recently (Wang et al., 2014).

Interestingly, Rictor KO CD8 T cells produce larger amounts of IL-2 compared with the WT during primary infection. At the memory phase, not only the frequency of IL-2-producing cells but also the intensity of IL-2 on a per-cell basis are largely increased (data not shown). These findings are in line with previous reports showing that autonomous IL-2 production by CD8 T cells during primary infection was critical for the subsequent mounting of an effective recall response (Feau et al., 2011; Williams et al., 2006).

In this regard, it remains to be addressed whether enhanced IL-2 production during a primary response in Rictor-deficient CD8 T cells accounts for the more potent recall response.

The effector function of Rictor-deficient CD8 T cells remained largely intact, as also shown in a recent study (Pollizzi et al., 2015). However, in contrast to that report showing an unchanged, if not enhanced, frequency of Rictor KO antigen-specific CD8 T cells, we observed a decreased accumulation of CD8 effector T cells with Rictor deficiency at the peak of the response to high- or low-affinity ligand-bearing *Listeria*. We discovered that this was mainly due to less sustained proliferation in the face of comparable apoptosis rates of WT or Rictor KO effectors. The discrepancy between the two studies may be due to different infection systems. Pollizzi et al. (2015) transferred 100-fold more OT-1 cells than we did and used *Vaccinia* instead of *Listeria* infection to convey the OVA antigen. Another difference is the use of slightly different Rictor-floxed mice in the two studies. Indeed, the Rictor-floxed mice from Pollizzi et al. (2015) were exon 3-flanked, whereas ours were exon 4- and 5-flanked (Bentzinger et al., 2008). Nevertheless, both Polizzi's report and our report clearly demonstrate that effector functions such as cytokine production or killing capacity are unchanged upon Rictor deficiency, whereas enhanced memory responses can be induced.

It has become clear that CD8 T cell effector or memory differentiation processes are coupled with metabolic reprogramming. Unlike naive or effector T cells, memory CD8 T cells show higher SRC. In our study, we show that mTORC2-deficient CD8 T cells show increased SRC and FAO even in the presence of IL-2, indicating that metabolic reprogramming prevails even under conditions conducive to effector cell T cell differentiation, such as supplementation with IL-2 in activated T cell culture medium. We believe that the enhanced SRC and FAO in Rictor-deficient T cells were, at least in part, due to decreased Akt activity caused by mTORC2 functional deficiency. Indeed, we observed that a short period of Akt inhibition in vitro during CD8 T cell priming leads to enhanced generation of T cells with the memory phenotype (data not shown). More importantly, these Akt inhibitor pre-treated CD8 T cells show increased SRC and FAO, as determined by Seahorse analysis. Interestingly, we observed a significant increase in CPT1α expression in either Rictor KO CD8 T cells or WT CD8 T cells cultured in the presence of Akt inhibitor. Clearly, further exploration of mitochondrial function in mTORC2-deficient CD8 T cell differentiation is warranted.

SGK1 represents another key target downstream of mTORC2 signaling. Recently, SGK1 has been shown to promote Th2 differentiation (Heikamp et al., 2014). However, the role of SGK1 in CD8 T cells is unclear. Therefore, it remains important to address the possibility of SGK1 involvement in the regulation of transcriptional activity of Foxo1 and in CD8 memory formation. Foxo1 is a transcription factor that regulates genes controlling multiple cellular processes, including metabolism. However, Foxo1 activity seems to be tightly regulated by the exact amount of nuclear Foxo1, the availability of its co-activators/repressors, and post-translational modifications. It is conceivable that there might be differential requirements for Foxo1 during memory T cell differentiation and maintenance, important aspects that require further scrutiny.

Inhibition of mTORC1 or Akt correlates with increased memory formation and long-term persistence in vivo and enhanced tumor protection effects (Araki et al., 2009; Crompton et al., 2015; Kim et al., 2012; Pearce et al., 2009). We identified mTORC2 as a critical regulator of CD8 memory formation by orchestrating transcriptional and metabolic programming. mTORC2 signaling has typically been associated with control of cytoskeleton organization and cell adhesion and motility. These aspects need to be probed in responding Rictor-deficient CD8 T cells. In CD8 T cells, however, its role as a repressor of Foxo1 transcriptional activity suggests that the level of mTORC2 signaling in activated T cells has a direct effect on their ability to undertake the memory T cell differentiation pathway. Together, our findings raise the possibility that therapeutic inhibition of mTORC2 represents an effective strategy to generate robust memory responses against infections and cancer and but preserve potent effector functions.

EXPERIMENTAL PROCEDURES

Animals

Rictor[flox/flox] mice have been described previously (Bentzinger et al., 2008). T cell-specific Rictor KO mice were obtained by crossing Rictor[flox/flox] mice with transgenic mice that carried CD4 promoter-mediated Cre recombinase. All mouse strains were backcrossed to the C57BL/6J background. Rictor KO mice were further crossed on the OT-1 background. CD45.1 congenic C57BL/6 mice and CD45.1/2 WT OT-1 mice were maintained at the University of Lausanne's specific pathogen-free facility. This study was approved by the veterinary authority of the Canton Vaud (Permit 2688.0), and all experiments were performed in accordance with Swiss ethical guidelines.

OT-1 Cell Transfer and LM-OVA Infection

Naive OT-1 cells were purified with a naive CD8 isolation kit (STEMCELL Technologies). 10,000 CD45.2 naive WT or Rictor KO OT-1 cells were then transferred into the naive CD45.1 host intravenously (i.v.). For co-transfer settings, 10,000 CD45.1/2 WT naive OT-1 cells were mixed at a 1:1 ratio with naive OT-1 cells either from CD45.2 Rictor KO mice or CD45.2 WT littermates. Within 24 hr after OT-1 cell transfer, 2000 colony-forming units (CFUs) of LM-OVA N4 or T4 variants were administered i.v.

More experimental procedures can be found in the Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.12.095.

AUTHOR CONTRIBUTIONS

L.Z. conceived the study, performed most of the experiments, analyzed the data, and wrote the manuscript. B.O.T performed the real-time PCR analysis and constructed the shRNA and Foxo1 AAA retroviral vectors. S.G.O and D.Z. provided LM-OVA N4 and T4. I.C.L.M. performed the Seahorse analysis. M.M. and G.S. helped with the infection and phenotype analysis. M.A.R. and M.N.H. provided the Rictor-floxed mice. L.F helped with the Seahorse analysis. A.D. performed the in vitro killing assay and participated, with J.P.M., in the initiation of the project and in the elaboration of the manuscript. P.R. conceived and supervised the study, interpreted the data, and wrote the manuscript.

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REFERENCES

Araki, K., Turner, A.P., Shaffer, V.O., Gangappa, S., Keller, S.A., Bachmann, M.F., Larsen, C.P., and Ahmed, R. (2009). mTOR regulates memory CD8 T-cell differentiation. Nature *460*, 108–112.

Bentzinger, C.F., Romanino, K., Cloëtta, D., Lin, S., Mascarenhas, J.B., Oliveri, F., Xia, J., Casanova, E., Costa, C.F., Brink, M., et al. (2008). Skeletal muscle-specific ablation of raptor, but not of rictor, causes metabolic changes and results in muscle dystrophy. Cell Metab. *8*, 411–424.

Betz, C., and Hall, M.N. (2013). Where is mTOR and what is it doing there? J. Cell Biol. *203*, 563–574.

Böttcher, J., and Knolle, P.A. (2015). Global transcriptional characterization of CD8+ T cell memory. Semin. Immunol. *27*, 4–9.

Chang, J.T., Wherry, E.J., and Goldrath, A.W. (2014). Molecular regulation of effector and memory T cell differentiation. Nat. Immunol. *15*, 1104–1115.

Chen, H., Zhang, L., Zhang, H., Xiao, Y., Shao, L., Li, H., Yin, H., Wang, R., Liu, G., Corley, D., et al. (2013). Disruption of TSC1/2 signaling complex reveals a checkpoint governing thymic CD4+ CD25+ Foxp3+ regulatory T-cell development in mice. FASEB J. *27*, 3979–3990.

Chi, H. (2012). Regulation and function of mTOR signalling in T cell fate decisions. Nat. Rev. Immunol. *12*, 325–338.

Crompton, J.G., Sukumar, M., Roychoudhuri, R., Clever, D., Gros, A., Eil, R.L., Tran, E., Hanada, K., Yu, Z., Palmer, D.C., et al. (2015). Akt inhibition enhances expansion of potent tumor-specific lymphocytes with memory cell characteristics. Cancer Res. *75*, 296–305.

Delgoffe, G.M., Kole, T.P., Zheng, Y., Zarek, P.E., Matthews, K.L., Xiao, B., Worley, P.F., Kozma, S.C., and Powell, J.D. (2009). The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity *30*, 832–844.

Delgoffe, G.M., Pollizzi, K.N., Waickman, A.T., Heikamp, E., Meyers, D.J., Horton, M.R., Xiao, B., Worley, P.F., and Powell, J.D. (2011). The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat. Immunol. *12*, 295–303.

Dudda, J.C., Salaun, B., Ji, Y., Palmer, D.C., Monnot, G.C., Merck, E., Boudousquie, C., Utzschneider, D.T., Escobar, T.M., Perret, R., et al. (2013). MicroRNA-155 is required for effector CD8+ T cell responses to virus infection and cancer. Immunity *38*, 742–753.

Feau, S., Arens, R., Togher, S., and Schoenberger, S.P. (2011). Autocrine IL-2 is required for secondary population expansion of CD8(+) memory T cells. Nat. Immunol. *12*, 908–913.

Heikamp, E.B., Patel, C.H., Collins, S., Waickman, A., Oh, M.H., Sun, I.H., Illei, P., Sharma, A., Naray-Fejes-Toth, A., Fejes-Toth, G., et al. (2014). The AGC kinase SGK1 regulates TH1 and TH2 differentiation downstream of the mTORC2 complex. Nat. Immunol. *15*, 457–464.

Hess Michelini, R., Doedens, A.L., Goldrath, A.W., and Hedrick, S.M. (2013). Differentiation of CD8 memory T cells depends on Foxo1. J. Exp. Med. *210*, 1189–1200.

Intlekofer, A.M., Takemoto, N., Wherry, E.J., Longworth, S.A., Northrup, J.T., Palanivel, V.R., Mullen, A.C., Gasink, C.R., Kaech, S.M., Miller, J.D., et al. (2005). Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. Nat. Immunol. *6*, 1236–1244.

Jeannet, G., Boudousquié, C., Gardiol, N., Kang, J., Huelsken, J., and Held, W. (2010). Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. Proc. Natl. Acad. Sci. USA *107*, 9777–9782.

Ji, Y., Pos, Z., Rao, M., Klebanoff, C.A., Yu, Z., Sukumar, M., Reger, R.N., Palmer, D.C., Borman, Z.A., Muranski, P., et al. (2011). Repression of the DNA-binding inhibitor Id3 by Blimp-1 limits the formation of memory CD8+ T cells. Nat. Immunol. *12*, 1230–1237.

Joshi, N.S., Cui, W., Chandele, A., Lee, H.K., Urso, D.R., Hagman, J., Gapin, L., and Kaech, S.M. (2007). Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. Immunity *27*, 281–295.

Kaech, S.M., and Cui, W. (2012). Transcriptional control of effector and memory CD8+ T cell differentiation. Nat. Rev. Immunol. *12*, 749–761.

Kaech, S.M., Tan, J.T., Wherry, E.J., Konieczny, B.T., Surh, C.D., and Ahmed, R. (2003). Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat. Immunol. *4*, 1191–1198.

Kallies, A., Xin, A., Belz, G.T., and Nutt, S.L. (2009). Blimp-1 transcription factor is required for the differentiation of effector CD8(+) T cells and memory responses. Immunity *31*, 283–295.

Kim, E.H., Sullivan, J.A., Plisch, E.H., Tejera, M.M., Jatzek, A., Choi, K.Y., and Suresh, M. (2012). Signal integration by Akt regulates CD8 T cell effector and memory differentiation. J. Immunol. *188*, 4305–4314.

Kim, M.V., Ouyang, W., Liao, W., Zhang, M.Q., and Li, M.O. (2013). The transcription factor Foxo1 controls central-memory CD8+ T cell responses to infection. Immunity *39*, 286–297.

Krishna, S., Yang, J., Wang, H., Qiu, Y., and Zhong, X.P. (2014). Role of tumor suppressor TSC1 in regulating antigen-specific primary and memory CD8 T cell responses to bacterial infection. Infect. Immun. *82*, 3045–3057.

Pearce, E.L., Walsh, M.C., Cejas, P.J., Harms, G.M., Shen, H., Wang, L.S., Jones, R.G., and Choi, Y. (2009). Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature *460*, 103–107.

Pollizzi, K.N., Patel, C.H., Sun, I.H., Oh, M.H., Waickman, A.T., Wen, J., Delgoffe, G.M., and Powell, J.D. (2015). mTORC1 and mTORC2 selectively regulate CD8+ T cell differentiation. J. Clin. Invest. *125*, 2090–2108.

Powell, J.D., Pollizzi, K.N., Heikamp, E.B., and Horton, M.R. (2012). Regulation of immune responses by mTOR. Annu. Rev. Immunol. *30*, 39–68.

Rao, R.R., Li, Q., Gubbels Bupp, M.R., and Shrikant, P.A. (2012). Transcription factor Foxo1 represses T-bet-mediated effector functions and promotes memory CD8(+) T cell differentiation. Immunity *36*, 374–387.

Rutishauser, R.L., Martins, G.A., Kalachikov, S., Chandele, A., Parish, I.A., Meffre, E., Jacob, J., Calame, K., and Kaech, S.M. (2009). Transcriptional repressor Blimp-1 promotes CD8(+) T cell terminal differentiation and represses the acquisition of central memory T cell properties. Immunity *31*, 296–308.

Sallusto, F., Lenig, D., Förster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature *401*, 708–712.

Sarkar, S., Kalia, V., Haining, W.N., Konieczny, B.T., Subramaniam, S., and Ahmed, R. (2008). Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. J. Exp. Med. *205*, 625–640.

Shimobayashi, M., and Hall, M.N. (2014). Making new contacts: the mTOR network in metabolism and signalling crosstalk. Nat. Rev. Mol. Cell Biol. *15*, 155–162.

Shin, J., Wang, S., Deng, W., Wu, J., Gao, J., and Zhong, X.P. (2014). Mechanistic target of rapamycin complex 1 is critical for invariant natural killer T-cell development and effector function. Proc. Natl. Acad. Sci. USA *111*, E776–E783.

Shrestha, S., Yang, K., Wei, J., Karmaus, P.W., Neale, G., and Chi, H. (2014). Tsc1 promotes the differentiation of memory CD8+ T cells via orchestrating the transcriptional and metabolic programs. Proc. Natl. Acad. Sci. USA *111*, 14858–14863.

Tejera, M.M., Kim, E.H., Sullivan, J.A., Plisch, E.H., and Suresh, M. (2013). FoxO1 controls effector-to-memory transition and maintenance of functional CD8 T cell memory. J. Immunol. *191*, 187–199.

van der Windt, G.J., Everts, B., Chang, C.H., Curtis, J.D., Freitas, T.C., Amiel, E., Pearce, E.J., and Pearce, E.L. (2012). Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 36, 68–78.

van der Windt, G.J., O'Sullivan, D., Everts, B., Huang, S.C., Buck, M.D., Curtis, J.D., Chang, C.H., Smith, A.M., Ai, T., Faubert, B., et al. (2013). CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. Proc. Natl. Acad. Sci. USA 110, 14336–14341.

Waickman, A.T., and Powell, J.D. (2012). mTOR, metabolism, and the regulation of T-cell differentiation and function. Immunol. Rev. 249, 43–58.

Wang, C., Qin, L., Manes, T.D., Kirkiles-Smith, N.C., Tellides, G., and Pober, J.S. (2014). Rapamycin antagonizes TNF induction of VCAM-1 on endothelial cells by inhibiting mTORC2. J. Exp. Med. 211, 395–404.

Wherry, E.J., Teichgräber, V., Becker, T.C., Masopust, D., Kaech, S.M., Antia, R., von Andrian, U.H., and Ahmed, R. (2003). Lineage relationship and protective immunity of memory CD8 T cell subsets. Nat. Immunol. 4, 225–234.

Williams, M.A., Tyznik, A.J., and Bevan, M.J. (2006). Interleukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells. Nature 441, 890–893.

Yang, C.Y., Best, J.A., Knell, J., Yang, E., Sheridan, A.D., Jesionek, A.K., Li, H.S., Rivera, R.R., Lind, K.C., D'Cruz, L.M., et al. (2011). The transcriptional regulators Id2 and Id3 control the formation of distinct memory CD8+ T cell subsets. Nat. Immunol. 12, 1221–1229.

Yang, K., Shrestha, S., Zeng, H., Karmaus, P.W., Neale, G., Vogel, P., Guertin, D.A., Lamb, R.F., and Chi, H. (2013). T cell exit from quiescence and differentiation into Th2 cells depend on Raptor-mTORC1-mediated metabolic reprogramming. Immunity 39, 1043–1056.

Zehn, D., Lee, S.Y., and Bevan, M.J. (2009). Complete but curtailed T-cell response to very low-affinity antigen. Nature 458, 211–214.

Zhang, L., Zhang, H., Li, L., Xiao, Y., Rao, E., Miao, Z., Chen, H., Sun, L., Li, H., Liu, G., and Zhao, Y. (2012). TSC1/2 signaling complex is essential for peripheral naïve CD8+ T cell survival and homeostasis in mice. PLoS ONE 7, e30592.

Zhang, L., Tschumi, B.O., Corgnac, S., Rüegg, M.A., Hall, M.N., Mach, J.P., Romero, P., and Donda, A. (2014). Mammalian target of rapamycin complex 1 orchestrates invariant NKT cell differentiation and effector function. J. Immunol. 193, 1759–1765.

Zhou, X., Yu, S., Zhao, D.M., Harty, J.T., Badovinac, V.P., and Xue, H.H. (2010). Differentiation and persistence of memory CD8(+) T cells depend on T cell factor 1. Immunity 33, 229–240.

Zhu, L., Yang, T., Li, L., Sun, L., Hou, Y., Hu, X., Zhang, L., Tian, H., Zhao, Q., Peng, J., et al. (2014). TSC1 controls macrophage polarization to prevent inflammatory disease. Nat. Commun. 5, 4696.