**LETTER**

**Metabolic heterogeneity underlies reciprocal fates of T<sub>H17</sub> cell stemness and plasticity**

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A defining feature of adaptive immunity is the development of long-lived memory T cells to curtail infection. Recent studies have identified a unique stem-like T-cell subset amongst exhausted CD8-positive T cells in chronic infection<sup>1–8</sup>, but it remains unclear whether CD4-positive T-cell subsets with similar features exist in chronic inflammatory conditions. Amongst helper T cells, T<sub>H17</sub> cells have prominent roles in autoimmunity and tissue inflammation and are characterized by inherent plasticity<sup>4–7</sup>, although how such plasticity is regulated is poorly understood. Here we demonstrate that T<sub>H17</sub> cells in a mouse model of autoimmune disease are functionally and metabolically heterogeneous; they contain a subset with stemness-associated features but lower anabolic metabolism, and a reciprocal subset with higher metabolic activity that supports reprogramming to T-cell activation and differentiation<sup>14</sup>, but metabolic pathways including cholesterol homeostasis and glycolysis. 

Metabolic heterogeneity underlies reciprocal fates of T<sub>H17</sub> cell stemness and plasticity in helper T cells.

We hypothesized that T<sub>H17</sub> cells in autoimmune microenvironments are heterogeneous and consist of subpopulations with differing extents of lineage stability and plasticity. In the transcriptome of T<sub>H17</sub> cells from mice with experimental autoimmune encephalomyelitis (EAE), Cd27 (ref. <sup>9</sup>) was more highly expressed in cells from draining lymph nodes than those from the central nervous system (CNS; Extended Data Fig. 1a). We used a fate-mapping system—crossing Il17aCre mice with animals expressing the R26R<sup>YFP</sup> reporter—in order to permanently mark those cells that had expressed interleukin (IL)-17 with yellow fluorescent protein (YFP)<sup>9</sup>. Following immunization with myelin oligodendrocyte glycoprotein peptide (MOG), CD27 showed a unique bimodal expression, with distinct CD27<sup>+</sup> and CD27<sup>−</sup> fractions observed amongst YFP<sup>+</sup> T<sub>H17</sub> cells (Extended Data Fig. 1b). As EAE progressed, the CD27<sup>+</sup> subset initially shrank in proportion and then stabilized (Extended Data Fig. 1c), and at the peak of disease it was detected mainly in lymphoid tissues (draining lymph nodes and spleen) but not the spinal cord (Fig. 1a). Amongst YFP<sup>−</sup> T<sub>H17</sub> cells isolated from mice at day nine post-immunization (used throughout this study, unless otherwise noted), the CD27<sup>+</sup> fraction expressed decreased levels of IL-17, interferon (IFN)-γ (Fig. 1b) and the proliferative marker Ki-67 (Extended Data Fig. 1d). After ex vivo MOG stimulation, CD27<sup>+</sup> cells proliferated and converted into CD27<sup>−</sup> cells, whereas CD27<sup>−</sup> cells remained CD27<sup>−</sup> (Fig. 1c). When transferred into naive hosts, a fraction of CD27<sup>−</sup> cells developed into CD27<sup>−</sup> cells, while CD27<sup>−</sup> YFP<sup>+</sup> cells remained CD27<sup>−</sup> (Extended Data Fig. 1e). Moreover, CD27<sup>−</sup> cells expressed high levels of T-cell factor (TCF)-1 and Bcl-2, proteins that mediate CD8<sup>+</sup>T cell memory<sup>10,11</sup> (Fig. 1d and Extended Data Fig. 1f). Persistence of CD27<sup>−</sup> cells was also enhanced when they were transferred into recipients lacking the Rag1 protein (which is required for lymphocyte development) (Fig. 1e). Therefore, CD27<sup>−</sup> cells are characterized by in vivo quiescence and persistence, and by the ability to develop into CD27<sup>−</sup> cells.

To explore the underlying cellular and functional processes, we performed transcriptome analysis of CD27<sup>−</sup> and CD27<sup>+</sup> cells (Extended Data Fig. 2a). Gene-set-enrichment analysis (GSEA) using effector and memory-precursor CD8<sup>+</sup> T cell signatures<sup>12</sup> and CD8<sup>−</sup> memory and T<sub>H17</sub> (follicular helper) overlapping signatures<sup>13</sup> suggested that the CD27<sup>−</sup> subset was highly enriched for memory-associated signatures, while the CD27<sup>−</sup> subset was enriched for genes that are related to effector cells or downregulated in memory cells (Fig. 1f and Extended Data Fig. 2b). The transcriptomes of CD27<sup>−</sup> and CD27<sup>+</sup> T<sub>H17</sub> cells also showed considerable similarity with those of memory-like CXCR5<sup>+</sup> and CXCR5<sup>−</sup> subsets, respectively, of exhausted CD8<sup>+</sup> T cells<sup>1–3</sup> (Fig. 1f and Extended Data Fig. 2b). Using ‘hallmark’ gene sets in GSEA, we found that the CD27<sup>−</sup> subset was enriched for hallmark signatures of apoptosis (Extended Data Fig. 2c), mTORC1 signalling, Myc targets, and metabolic pathways including cholesterol homeostasis and glycolysis (Extended Data Fig. 2d). Consistent with these results, mTORC1 activity and Myc expression were higher in CD27<sup>−</sup> than in CD27<sup>+</sup> T<sub>H17</sub> cells (Fig. 1g, h). Moreover, blocking glycolysis with 2-deoxyglucose (2-DG) increased CD27 expression in YFP<sup>+</sup> cells (Fig. 1i), indicating stabilization of the CD27<sup>+</sup> T<sub>H17</sub> phenotype. Overall, T<sub>H17</sub> cells are heterogeneous and consist of two subpopulations, with the CD27<sup>−</sup> subset showing memory-like features and metabolic quiescence.

Emerging studies have highlighted the importance of metabolic reprogramming to T-cell activation and differentiation<sup>14</sup>, but metabolic control of cellular plasticity is unclear. We therefore determined the effects on T<sub>H17</sub> heterogeneity and plasticity of blocking mTORC1 activity by using the Il17aCre and R26R<sup>YFP</sup> system<sup>9</sup> to delete the signature mTORC1 component Raptor (encoded by Rptor). Naïve T cells from wild-type and Rpto<sup>Il17aCre</sup> mice showed comparable proliferation and T<sub>H17</sub> differentiation (Extended Data Fig. 3a). However, Rpto<sup>Il17aCre</sup> mice were protected from MOG-induced EAE (Fig. 2a), and exhibited lack of CNS inflammation and T-cell infiltration (Fig. 2b and Extended Data Fig. 3b). Raptor-deficient YFP<sup>+</sup> cells from the CNS upregulated IL-17 but were defective in IFN-γ expression (Fig. 2c). Thus, mTORC1 function, beyond initial T<sub>H17</sub> differentiation, is crucial for EAE and robust IFN-γ expression.

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CD27+ T H17 cells have memory-like features and low metabolic activity. a, Summary of CD27 expression on CD4+ TCR-β+ YFP+ cells at day 16 after MOG immunization in draining lymph nodes (dLN), spleen and spinal cord of Il17aCre (R26R-EGFP) mice (n = 8, dLN; n = 12, spleen and spinal cord). b−h, Analysis of CD27+ and CD27− YFP+ populations (b, left) from Il17aCre (R26R-EGFP) mice at day nine after MOG immunization. b, Centre and right panels, IL-17 and IFN-γ expression (n = 6, CD27+/CD27− IL-17; n = 8, CD27− IFN-γ; n = 9, CD27− IFN-γ). c, In vitro culture with MOG for analyses of proliferation (CellTrace Violet) and CD27 expression. d, TCF-1 expression in CD27+ and CD27− cells (left) and fold change (right; expression in CD27+ population was set to 1) (n = 9), e, CD27+ or CD27− YFP+ cells were transferred into Rag1−/− mice, and analysed at day 15 for donor-cell percentages (left) and numbers (right, normalized against cell numbers at day 1) (n = 3, CD27+).

Because IL-17 can be produced by cells other than T H17 cells, we constructed mixed bone-marrow chimaeras to restrict Raptor deficiency to α/β T H17 cells (Extended Data Fig. 3c). RaptorIl17aCre-derived chimaeras were resistant to EAE (Extended Data Fig. 3c), indicating the role of mTORC1 in T H17 cells selectively. Additionally, to exclude the effects of absent inflammation in RaptorIl17aCre mice, we generated mixed chimaeras using CD45.2+ RaptorIl17aCre (or wild-type control) and CD45.1+ wild-type bone-marrow-derived cells, which would mediate CNS inflammation in EAE (Extended Data Fig. 3d). Following MOG immunization, Raptor-deficient cells showed reduced cellularity in CNS, with a preferential loss of IL-17+ IFN-γ− cells (Extended Data Fig. 3e, f). Raptor-deficient cells also had reduced expression of Ki-67 and T-bet but normal survival and expression of the CCR6 and CXCR3 chemokine receptors (Extended Data Fig. 3g−k). These results identify an intrinsic requirement for mTORC1 in mediating IFN-γ production and in sustaining T H17 responses at the site of inflammation.

In draining lymph nodes from MOG-immunized RaptorIl17aCre (R26R-EGFP) mice, we found a modestly lower percentage of YFP+ cells, which showed efficient Raptor deletion and diminished mTORC1 activity (Extended Data Fig. 4a, b). Raptor-deficient YFP+ cells exhibited normal survival, chemokine receptors and IL-17 expression, but produced less IFN-γ (Extended Data Fig. 4c−e). Moreover, Raptor-deficient cells had reduced expression of T-bet, Tbx21 and Il12rb2, normal expression of ROR-γ and Foxp3, and elevated expression of RorC and Il23r (Fig. 2d) and Extended Data Fig. 4f, g). Thus, loss of Raptor in T H17 cells impairs expression of T H11-associated factors. In response to ex vivo MOG stimulation, Raptor-deficient T H17 cells produced much less IFN-γ and modestly more IL-17 (Fig. 2e), with largely unaffected proliferation (Extended Data Fig. 4h). Addition of IL-12 converted many IL-17-producing cells into IL-17+ IFN-γ− cells, but Raptor-deficient cells were less capable of such conversion or transdifferentiation (Fig. 2f). Conversely, IL-23 induced a predominant IL-17+ IFN-γ− population in a Raptor-dependent manner (Extended Data Fig. 4i). Whereas both cytokines induced IFN-γ expression, IL-23 but not IL-12 maintained IL-17 expression, suggesting a more complete T H1 transdifferentiation induced by IL-12. Furthermore, Raptor-deficient cells were impaired in IL-12-induced phosphorylation of the

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transcription factor STAT4, but showed only a small defect in IL-23-induced STAT3 phosphorylation (Extended Data Fig. 4j). Altogether these results show that mTORC1 facilitates the transdifferentiation of T\textsubscript{H}17 cells into IFN-\(\gamma\)-producing cells with T\textsubscript{H}1-like features after antigen stimulation, and that this can be intensified by cytokine signals from IL-12 and, to a lesser extent, IL-23.

Transcriptome and functional enrichment analyses of Raptor-deficient T\textsubscript{H}17 cells identified the acetylation and cholesterol-biosynthesis pathways as amongst the most extensively downregulated (Extended Data Fig. 5a). mTORC1 signalling, Myc targets and metabolic pathways were also attenuated (Extended Data Fig. 5b). Moreover, Raptor-deficient and CD27\textsuperscript{+} (versus CD27\textsuperscript{−}) T\textsubscript{H}17 cells shared downregulated metabolic pathways, including Myc and mTORC1 signalling and nucleotide metabolism (Extended Data Fig. 5c, d); upregulated or downregulated expression changes in CD27\textsuperscript{+} T\textsubscript{H}17 cells were positively correlated with those of Raptor-deficient or wild-type cells, respectively (Extended Data Fig. 5e). In further support of mTORC1 signalling as a differentiating feature of CD27\textsuperscript{+} and CD27\textsuperscript{−} cells, CD27 expression was upregulated in Raptor-deficient cells (Extended Data Fig. 5f).

We next used Ingenuity pathway analysis (IPA) to identify transcription factors that mediate mTORC1 function, and identified downregulation of Myc, sterol regulatory element-binding proteins (SREBPs) and STATs in Raptor-deficient T\textsubscript{H}17 cells (Extended Data Fig. 6a). Flow cytometry validated the lower Myc expression in Raptor-deficient cells (Extended Data Fig. 6b). To explore the metabolic dependence of T\textsubscript{H}17 fates, we used the Il17aCre and R26\textsuperscript{ERT2} systems to delete Myc (Extended Data Fig. 6c, d) or Hmgcr, which encodes a SREBP-dependent rate-limiting enzyme for cholesterol biosynthesis (Extended Data Fig. 6e, f). T\textsubscript{H}17 cells from these mice largely phenocopied Raptor-deficient cells, as exemplified by reduced Tbx21 and Il12rb2 expression (Extended Data Fig. 6c, e) and an impaired ability to transdifferentiate into IFN-\(\gamma\)-expressing cells (Extended Data Fig. 6d, f). Moreover, deletion of Myc, Hmgcr or Raptor all upregulated expression of CD27 on T\textsubscript{H}17 cells (Extended Data Fig. 6g). Finally, to gain insight into pathways

### Figure 3: TCF-1 expression is regulated by mTORC1 and metabolic activity in T\textsubscript{H}17 cells

**a–d**, ATAC-seq analysis of YFP\textsuperscript{+} cells isolated from the draining lymph nodes (dLN) and spleen of WT and Rptor\textsuperscript{Il17aCre} (R26\textsuperscript{ERT2}) mice, or the spleen of R26\textsuperscript{ERT2} mice treated with phosphate-buffered saline (PBS) vehicle or 2-DG at day nine post MOG-immunization. **a**, Principal component analysis (PCA) plot of nucleosome-free fragments. **b**, Accessibility of the Ifng locus in different animals, aligned with T-bet-binding sites (red boxes show promoter regions). The bottom four rows show T-bet-binding signals in the indicated public database (WCE, whole-cell extract, control sample without immunoprecipitation). **c**, Motif-enrichment analysis of binding profiles of selected transcription factors from the TCF–LEF family. ‘M’ numbers refer to TRANSFAC accession numbers for the respective motif sequences. **d**, Flow cytometry of T-bet and TCF-1 expression in freshly isolated YFP\textsuperscript{+} cells from dLN of mice at day nine after MOG immunization (e), or after in vitro stimulation with MOG plus IL-12 for four days (f). **g**, Plot of odds ratio versus odds ratio for motif-enrichment data from Rptor\textsuperscript{Il17aCre} versus WT and 2-DG versus PBS samples. **h**, Flow cytometry of T-bet and TCF-1 expression in YFP\textsuperscript{+} cells from Il17aCre (R26\textsuperscript{ERT2}) mice cultured with MOG plus IL-12, and vehicle or 2-DG (1 mM). Data are representative of one (a–d, g), four (e, f) or three (h) independent experiments.

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downstream of mTORC1 signalling, we used inhibitors of the S6K and 4E-BP branches. We found that drugs targeting 4E-BP but not S6K signalling impaired the transition of \( T_{17} \) cells into IFN-\( \gamma \)-producing cells, although both pathways contributed to cell proliferation (Extended Data Fig. 6h, i). Altogether, our transcriptomic and functional perturbation studies indicate that interplay between mTORC1 signalling, especially the 4E-BP branch, and metabolic reprogramming controls \( T_{17} \) cell plasticity.

Consistent with downregulation of acetylation in Raptor-deficient cells (Extended Data Fig. 5a), we found impaired binding of acetyl histone H3 to the \( \gamma\)-IFN (IFN-\( \gamma \) gene) promoter (Extended Data Fig. 7a). Also, ATAC-seq (assay for transposable accessible chromatin using sequencing) (Fig. 3a and Extended Data Fig. 7a, b) showed that Raptor-deficient cells had less accessibility in the \( \gamma\)-IFN promoter, corresponding to T-bet-binding sites (Fig. 3b), and in the promoters for \( T_{b}21 \) and \( \gamma\)-IFN (Extended Data Fig. 7d). Searches for transcription-factor binding motifs in accessible regions of ATAC-seq reads revealed enriched occupancy of factors from the TCF–LEF family in Raptor-deficient cells (Fig. 3c, d, Extended Data Fig. 7e, f). Next we carried out in-depth footprint analysis, in which we superimposed our ATAC-seq results with publicly available TCF-1 chromatin immunoprecipitation (ChIP)-seq data sets. We identified TCF–LEF-binding motifs in \( R_{p}t0^{1l7aCRE} \) but not in wild-type open-chromatin regions (Extended Data Fig. 8a); by contrast, we found many more T-bet-binding motifs in the wild-type open-chromatin regions (Extended Data Fig. 8a). To validate the observation of enhanced TCF-1 binding to target genes in Raptor-deficient \( T_{17} \) cells, we chose two candidate genes—\( I_{6}f_{a}a \) and \( L_{ri}g^{1/16} \) (Extended Data Fig. 8b)—for ChIP assays, and found much greater binding of TCF-1 to these genes in Raptor-deficient cells than in wild-type cells (Extended Data Fig. 8c).

Moreover, co-staining of TCF-1 and T-bet in \( T_{17} \) cells identified two reciprocal populations, with an increased TCF-1\( ^{b}T_{b}21^{b} \) but a reduced TCF-1\( ^{b}T_{b}21^{b} \) population upon loss of Raptor (Fig. 3e). Following culture with MOG and IL-12, wild-type cells became almost exclusively TCF-1\( ^{b}T_{b}21^{b} \), while Raptor deficiency reduced this transition (Fig. 3f). Similarly, Myc deletion increased the TCF-1\( ^{b}T_{b}21^{b} \) population at the expense of the TCF-1\( ^{b}T_{b}21^{b} \) subset (Extended Data Fig. 8d). Altogether, TCF–LEF factors are enriched in the genomic landscape of Raptor-deficient \( T_{17} \) cells, and reciprocal expression of T-bet and TCF-1 in \( T_{17} \) cells depends upon mTORC1.

To directly assess the involvement of metabolism, we performed ATAC-seq analysis of \( T_{17} \) cells isolated from MOG-immunized mice treated with 2-DG (Fig. 3a). Remarkably, 2-DG-treated samples, like Raptor-deficient cells, showed reduced chromatin accessibility at the \( \gamma\)-IFN, \( T_{b}21 \) and \( \gamma\)-IFN promoters (Fig. 3b and Extended Data Fig. 7d), and enriched occupancy of TCF–LEF factors (Fig. 3g and Extended Data Fig. 8e). Furthermore, 2-DG treatment impaired the generation of the TCF-1\( ^{b}T_{b}21^{b} \) population (Fig. 3h) and IFN-\( \gamma \) expression in \( T_{17} \) cells in vitro and in vivo (Extended Data Fig. 8f, g). These results support the metabolic dependence of chromatin accessibility and reciprocal T-bet and TCF-1 expression in \( T_{17} \) cells.

We next used single-cell RNA sequencing (scRNA-seq) to dissect cellular heterogeneity in an unbiased manner. Wild-type and Raptor-deficient \( T_{17} \) cells had largely distinct distribution patterns (Fig. 4a and Extended Data Fig. 9a–c). Functional enrichment analysis on the differentially expressed genes within each cluster revealed the underlying cellular processes associated with each subpopulation (Extended Data Fig. 9d). Clusters composed mainly of Raptor-deficient cells were enriched with memory-associated gene signatures\(^{1–3,13} \), while wild-type-dominant clusters were reciprocally enriched with genes downregulated in memory cells (Fig. 4b and Extended Data Fig. 9e). To explore potential differences in temporal activation, we superimposed our scRNA-seq data with published data sets from CD8\( ^{+} \) memory T cells stimulated once versus multiple times\(^{18} \), respectively designated as ‘early memory’ (less mature) and ‘late memory’ (terminal differentiation) signatures\(^{19} \). Raptor-deficient and wild-type cells were enriched with ‘early memory’ and ‘late memory’ gene signatures, respectively (Fig. 4c and Extended Data Fig. 9e). Moreover, enrichments for T-bet target genes and glycolysis were mainly observed in wild-type-dominant clusters (Extended Data Fig. 9e, f), whereas \( R_{p}t0^{1l7aCRE} \)-dominant clusters showed increased expression of memory-associated genes \( B_{2}1 \), \( D_{2}7^{\gamma} \) and \( T_{c}f^{7} \) (refs.\(^{9–11} \) Extended Data Fig. 9g). Therefore, single-cell transcriptomics reveals a marked heterogeneity of \( T_{17} \) cells, with reciprocal expression of stemness-like and terminal-differentiation signatures, and highlights mTORC1-dependent shaping of these heterogeneous features.

To reconstruct developmental trajectory, we analysed our scRNA-seq data using Monocle 2 for pseudotemporal ordering of cells (pseudotime)\(^{20} \). Using a group of highly expressed and dispersed genes (Extended Data Fig. 10a), we derived a relative trajectory with visualisation of ‘early memory’ and ‘late memory’ gene signatures.\(^{18,19} \)
pronounced differences in the pseudotime assignment of the nine clusters (Fig. 4d and Extended Data Fig. 10b). Projection of gene signatures onto the pseudotime trajectory revealed the assignment of ‘early memory’ and ‘late memory’ signatures with early and late pseudotime, respectively, corroborating our analysis (Extended Data Fig. 10c). The ‘T-bet targets’ signature (Extended Data Fig. 10c) and Tbx21 and Ifng expression (Extended Data Fig. 10d, e) were restricted to branches of late pseudotime, suggesting late acquisition of T117 features. In addition, Raptor-deficient T1I7 cells had an increased propensity for early pseudotime assignment (Fig. 4e and Extended Data Fig. 10f), suggesting altered pseudotemporal ordering. Moreover, Cd27 and Tcf7 were expressed primarily in early pseudotime and associated with Raptorα1I7αCre dominant clusters (Extended Data Fig. 10g, h). These analyses suggest a role for mTORC1 in promoting the terminal differentiation of T1I-like cells from CD27⁺ T1I7 cells.

Although freshly isolated CD27⁺ T1I7 cells expressed low levels of IFN-γ and T-bet (Fig. 1b and Extended Data Fig. 10i), they acquired the ability to express these proteins to a similar extent as CD27⁻ cells after antigen stimulation (Fig. 4f, g), indicating a much greater induction than in CD27⁻ cells (Extended Data Fig. 10j). To further examine the role of mTORC1 in this process, we isolated CD27⁺ or CD27⁻ YFP⁺ cells from wild-type or Raptorα1I7αCre mice and transferred them into recipients, which were subsequently immunized with MOG. Although a substantial number of wild-type CD27⁻ cells became CD27⁺, most Raptor-deficient cells retained their CD27 expression (Extended Data Fig. 10k), indicating the arrested differentiation of CD27⁻ into CD27⁺ cells. Moreover, Raptor-deficient CD27⁻ cells were less efficient at transitioning into CD27⁺ cells after in vitro MOG and IL-12 stimulation (Extended Data Fig. 10l). Altogether, these functional data further support our hypothesis that mTORC1 drives the transition of CD27⁺ cells to CD27⁻ cells.

Our findings suggest that T1I7 responses in chronic autoimmune disease are phenotypically, transcriptionally and metabolically heterogeneous, encompassing a CD27⁺ TCF-1αβ subclass with inferred stemness features and low anabolic metabolism, and a reciprocal CD27⁻ T-betαβ subclass (Extended Data Fig. 10m). The CD27⁺ TCF-1αβ subclass can develop into the terminally differentiated CD27⁻ T-betαβ subpopulation with robust IFN-γ expression, but this transition is arrested upon mTORC1 deletion or metabolic perturbation. These results highlight that metabolic heterogeneity of T1I7 cells underlies lineage stability and plasticity, and point to a causative effect of metabolic reprogramming on these late developmental processes. Our results suggest that TCF-1 expression is sensitive to altered anabolic metabolism and may serve as a metabolic gatekeeper to preserve lineage identity and the stability of effector T cell lineages. From a therapeutic perspective, much as targeting cancer stem cells is an important goal for successful anticancer therapy, the identification of stem-like T1I7 cells opens up new avenues for therapeutic intervention in chronic inflammatory conditions.

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Author contributions P.W.F.K. conceived, designed and performed in vitro and in vivo experiments and bioinformatic analyses, analysed data, and wrote the manuscript. X.C. performed scRNA-seq analysis and developed the latent cellular state analysis (LCA) algorithm. S.A.L., A.A.H. and T.-L.M.N. helped to conceive and design experiments, co-wrote the manuscript, and provided overall direction.

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Mice. Mice were housed and bred at the St Jude Children's Research Hospital. For animal-care facilities in specific pathogen-free conditions. C57BL/6, Tcrα−−, CD45.1−, Il17aCre, Rag1−− and 2D2-transgenic mice were purchased from the Jackson Laboratory. Rtporβ, Mryβ and Hmgorβ mice were as described16-23. Cre-expressing mice were used as controls, and littermates were used when possible. Mice were backcrossed for at least ten generations to the C57BL/6 background strain. Female and male mice were used at 6–10 weeks of age. Sample sizes were selected to maximize the chance of uncovering mean differences that were also statistically significant. Age- and sex-matched mice were assigned randomly to experimental and control groups. The assessment of EAE scores and histopathology examination was performed in a blinded fashion. For bone-marrow chimeras, Tera−− mice were sublethally irradiated for a total of 500 Rads before receiving three million CD90.2− depleted bone-marrow donor cells. Mice remained on antibiotic (Baytril) water for 2–3 weeks, and, after 6 weeks, reconstitution was determined by flow cytometry analysis of blood samples. Mice were used 6–8 weeks after chimerism generation for experiments. All experiments with mice were conducted in accordance with the St Jude Children's Research Hospital institutional policies, and animal protocols were approved by the Institutional Animal Care and Use Committee of St Jude Children's Research Hospital.

EAE induction. Mice were immunized subcutaneously at four injection sites with a total of 200 μl of emulsified incomplete Freund's adjuvant supplemented with 1 mg Mycobacterium tuberculosis strain H37Ra (Difco) (complete Freund's adjuvant; CFA) and 200 μg myelin oligodendrocyte glycoprotein (amino acids 35–55; MOG35–55), and received intraperitoneal injections of 200 ng pertussis toxin (PTX; List Biological Laboratories) at the time of immunization and 48 h later. Mice were observed daily for clinical signs and scored as follows: normal mouse; 0, no overt signs of disease; 1, limp tail; 2, limp tail plus hindlimb weakness; 3, total hindlimb paralysis; 4, hindlimb paralysis plus 75% body paralysis (forelimb paralysis); 5, moribund.

FACS, antibodies and reagents. For analysis of surface markers, cells were stained in PBS (Gibco) containing 2% (w/v) bovine serum albumin (BSA; Sigma). Surface proteins were stained for 30 min on ice. CellTrace Violet labelling was performed according to the manufacturer's instructions (Thermo Fisher). Intracellular staining was performed on sorted YFP+ cells with the Foxp3 translocation factor staining buffer set according to the manufacturer's instructions (eBioscience). Intracellular staining for cytokines was performed with a fixation/permeabilization kit (BD Biosciences). Caspase-3 staining was performed using instructions and reagents from the 'active caspase-3 apoptosis kit' (BD Biosciences). We used 7-aminoactinomycin D (7-AAD; Sigma) for dead-cell exclusion at 2.5 μg/ml.

The following antibodies were used: anti-CD27 (LG.77F9), anti-CD45.2 (104), anti-IFN-γ (XMg1.2), anti-KLRG1 (2F1), anti-T-bet (eBio4B10) (all from eBioscience); anti-CD43 (activation glycoform; B220), anti-CD44 (IM7), anti-CD62L (MEL-14), anti-CD127 (A7R34), anti-CCR6 (HK1.4), anti-CD45.1 (A20), anti-CD62L (MEL-14), anti-CD127 (A7R34), anti-B220 (C11-B7) from Biolegend; anti-CD95 (Jo2), anti-Foxp3 (FJK-16s), anti-ROR-γt (HK1.4), anti-PD-1 (29F.1A12), anti-Sca-1 (D7), anti-TCR-β (H57-597) (all from BD Biosciences); anti-CD3 (17-2), anti-CD4 (G043-2), and anti-TCR-β (H57-597) with a protease-inhibitor cocktail (Roche) for 10 min on ice, before sonication into pieces of a maximum of 500 base pairs (bp) using a Diagenode Biorupter. Sheared chromatin was cleared of debris and incubated with either normal IgG (Cell Signaling Technology; 1:50) or anti-pan acetyl histone H3 (Millipore; 1:50), or anti-TCF-1 (Cell Signaling Technology; 1:50), and blocker (Active Motif) rotating overnight at 4°C. Chromatin immunoprecipitation and subsequent DNA purification was performed using the ChIP-IT high sensitivity kit (Active Motif) as per the manufacturer's instructions. We used the following qPCR primers, labelled with the DNA dye Sybr green: Ifng promoter F-GAGCCTAAAGGATGCAGAAGAAG; Ifng promoter R-CTAGGTGCACCGGTAGCAGCTA; Il6ra F-CTACGGTTTCTACTGAGTGGCCT; Lrig1 F-TAGGGCTGAGCTACTTAGA; Lrig1 R-CTGAATTCGCTTTGACAGTGG; negative control primer set (Active Motif catalogue number 71011) and positive control primer set (Active Motif number 71017). Data analysis was performed using the 'percent input' normalization method and displayed as a percentage of the WT control. To identify additional target genes aside from known TCF-1 target genes, we computationally identified genes whose genomic regions became more accessible in ATAC-seq data as a result of Raptor deletion. Using this subset of genes, we further compared microarray data from Raptor-deficient and WT cells and identified Lrig1 as a top candidate.

In vivo cell transfer. CD27+ or CD27− fringes of YFP+ cells were sorted after enrichment with CD4 beads (Miltenyi) from spleens and lymph nodes of MOG-immunized WT or Rptorfl/fl and 2-DG treated mice. Sorting was performed on a Reflection (i-Cyt) cytometer, and sort-purified cells were transferred via retroorbital injection into Rag1−− mice. Because of low cell numbers, we used day 1 after transfer as the baseline reading against which to normalize subsequent analyses at days 14–15, as described27, for donor-cell abundance in the spleen and lymph nodes. In vivo transfer experiments were performed using CD45.1+ hosts, except that 2D2-transgenic mice (expressing MOG-antigen-specific T-cell receptors2; crossed onto the Il17aCre (R26RFLYFP) fate-mapping system) were used as donor mice, and CD4+ T-cell enrichment was performed at the time of analysis, to facilitate flow cytometric detection of the rare population of donor cells.

Statistical analysis for biological experiments. For biological experiment (non-omics) analyses, we calculated P-values by two-way ANOVA, Student's t-test (two-sided) for parametric data or Mann–Whitney U-test (two-sided) for non-parametric data. Differences between groups were considered statistically significant with a P-value cut-off of 0.05 or less. Data are represented as means ± s.e.m. Graph Pad Prism (v. 6.0) was used to perform these statistical analyses.

Microarray analysis. Mice were immunized with MOG as described above. TCR-β+ YFP+ cells from dLN of WT or Rptorfl/fl (R26RFLOYP) mice or TCR-β+ YFP+ CD44+Il6ra+ and TCR-β+ YFP+ CD44+Il6ra+− T cell populations from dLN of Il17aCre (R26RFLOYP) mice at day nine post-immunization were analysed with the Affymetrix Mouse Gene 2.0 ST GeneChip array29, and expression signals were summarized with the robust multi-array average algorithm (Affymetrix Expression Console v1.1) or by fitting a linear model implemented in the R package 'limma'30. Lists of differentially expressed genes by 0.5 log, fold change or more were analysed using Ingenuity pathway analysis (Ingenuity.com) to identify underlying Ingenuity canonical pathways and upstream transcription regulators. Gene-set-enrichment analysis (GSEA) within 'hallmark’ gene sets was performed as described31. For GSEA using manually curated gene signatures from public data sets, refer to the section on 'Analysis for scRNA-seq data' below. Upregulated and downregulated genes under each condition were annotated using hallmark and KEGG gene sets (version 6.0 downloaded from MsigDB32), and functional enrichment of specific pathways in the gene sets was performed using Fisher's exact test. Fisher's exact P-value was corrected for multiple testing using the Benjamini–Hochberg (BH) method. Pathways were deemed significantly enriched at an FDR of less than 5% and enrichment score of 10 or more. To determine the empirical cumulative distribution of the test statistic, the Kolmogorov-Smirnov test statistic was calculated. These data have been deposited into the National Center for Biotechnology Information (NCBI)'s Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE107521.

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Preparation of the ATAC-seq library. Mice were immunized with MOG as described above. TCR-3' YFP' cells were sorted from the dLN or spleen at day nine post-immunization, and lysed in 50 μL ATAC-seq lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.1% IGEPA CA-630 detergent) on ice for 10 min. Resulting nuclei were pelleted at 500g for 10 min at 4°C. Supernatant was carefully removed with a pipette and discarded. The pellet was resuspended in 50 μL transposase reaction mix (25 μL 2× TD buffer, 22.5 μL nuclease-free water, 2.5 μL transposase) and incubated for 30 min at 37°C. After the reaction, the DNA was cleaned up using the Qiagen MinElute kit. The barcoding reaction was run using the NEBNext HIFI kit on the basis of the manufacturer’s instructions and amplified for five cycles according to ref. 15, using the same primers. Ideal cycle numbers were determined from 5 μl (of 50 μl) from the previous reaction mix using KAPA SYBRFast (KAPA Biosystems) and 20-cycle amplification on an Applied Biosystems 7900HT. Optimal cycle numbers were determined from the linear part of the amplification curve and the remaining 45 μl of PCR reaction were amplified in the same reaction mix using the optimal cycle number.

ATAC-seq analysis. We obtained 2 × 100 bp paired-end reads from all samples, removed any possible Nextera adapter sequences from the 3’ ends of the reads using cutadapt (version 1.9, paired-end mode, default parameter with ‘-m 6 -O 20’), and aligned them to the mouse genome mm9 (NCBI37_um from Sanger; ftp://ftp-mouse.sanger.ac.uk/refs/NCBI37_um.fa) using the Burrows–Wheelers algorithm12 (version 0.5.9-r26-dev, default parameter); duplicated reads were then marked with Picard (version 1.65 (1160)) and only non-duplicated reads were kept, using samtools (parameter ‘-q 1 -P 1024’ version 0.1.8 (r892:295)). After adjustment using the TiN shift (by which reads were offset by +4 bp for the sense strand and −5 bp for the antisense strand), we separated nine-nucleotide, mononucleosome, dinucleosome and trinucleosome by fragment size11, and generated bigwig files by using the centre 80 bp of fragments and scaling them to 30 M nucleosome-free reads. We observed reasonable nucleosome-free peaks and patterns of mononucleosomes, dinucleosomes and trinucleosomes on the Integrated Genomics Viewer15 (version 2.3.40), and visualized these using heat maps and aggregation plots centred by transcription start site (TSSs; Extended Data Fig. 7b). All samples had more than 20 M nucleosome-free reads, and visual inspection indicated adequate data quality (see Extended Data Fig. 7b), so we performed peak-calling for nucleosome-free reads using MACS213 (version 2.1.0.20150603, default parameters with ‘-extsize 200 –nomodel, merged by bedtools’ if within 100 bp) for individual samples. To ensure replicability, we first finalized nucleosome-free regions for each tissue or genotype, only retaining a called peak if it appeared in more than half of replicates; we then counted nucleosome-free reads from each replicate and drew correlation plots and distributions (Extended Data Fig. 7c). The Pearson correlation coefficients (all greater than 0.95) indicated that our data were highly reproducible across samples. Then we merged finalized nucleosome-free regions from all tissue/genotype iterations, counted nucleosome-free reads, and clustered them according to the samples separated well by genotype (Fig. 3a and Extended Data Fig. 7c).

To find the differentially accessible regions, we first normalized raw nucleosome-free read counts using the trimmed mean of M-values normalization method, and applied the empirical Bayes statistics test after linear fitting from the voom package36 (R 3.23, edgeR 3.12.1, limma 3.26.9). We used FDR-adjusted p-values of 0.05 as the cut-off for more-accessible regions or less-accessible regions (log2 fold change > 1). We further annotated the GEO under accession number GSE52070 and T-bet peaks were downloaded from GSM989272. For each group, differentially accessible peaks were overlapped with TCF-1 or T-bet peaks in order to identify regions that were common between ATAC-seq peaks and ChIP-seq peaks using bedtools (version 2.25.0). Finally, we used FIMO39 from the MEME suite (version 4.9.0) to scan the overlapping regions with TRANSFAC motifs for LEF–TCF-1 family members or T-bet, and Fisher’s exact test to test the significance of enrichment of motifs in the KO_Larger versus KO_Smaller regions described above.

scRNA-seq. Mice were immunized with MOG as described above. TCR-3' YFP' cells from spleen at day nine post-immunization were sorted on an l-1 Cry Reflection Cell Sorter in 20-mm tubes containing complete media, counted and placed on ice. Some samples were pooled before library construction in order to load sufficient cellular materials into the Chromium Controller instrument (10x Genomics)40. The cells were counted and examined for viability using a Luna dual fluorescence cell counter (Logos Biosystems). All samples were spun down at 2,000 r.p.m. for 5 min. The supernatant was removed, and cells were resuspended in 100 μl of 1 × PBS (Thermo Fisher Scientific) plus 0.04% BSA (Amresco). The cells were again counted and checked for viability using a Luna dual fluorescence cell counter (Logos Biosystems). Cell counts ranged from 4 × 10⁷ to 1.5 × 10⁸ cells per milliliter and viability was greater than 98%. Single-cell suspensions were loaded onto the Chromium Controller according to their respective cell counts to generate 6,000 single-cell gel beads in emulsion (GEMs) per sample. Each sample was loaded into a separate channel. Libraries were prepared using the Chromium single cell 3' v2 library and gel bead kit (10x Genomics). The complementary DNA content of each sample after cDNA amplification of 12 cycles was quantified and quality checked using a high-sensitivity DNA chip with a 2100 Bioanalyzer (Agilent Technologies) to determine the number of PCR amplification cycles to yield sufficient library for sequencing. After library quantification and quality checking by DNA 1000 chip (Agilent Technologies), sample libraries were diluted to 3.5 M for loading onto the HiSeq 4000 (Illumina) with a 2 × 75 paired-end kit using the following read length: 26 bp read1, 8 bp i7 index, and 98 bp read2. An average of 400,000,000 reads per sample was obtained (approximately 80,000 reads per cell).

Analysis of scRNA-seq data. Alignment, barcode assignment and unique molecular identifier (UMI) counting. The Cell Ranger 1.3 Single-Cell software suite (10x Genomics) was implemented to process the raw sequencing data from the Illumina HiSeq run. This pipeline performed demultiplexing, alignment (using the mouse genome mm10 from ENSEMBL GRCm38), and barcode processing to generate gene-cell matrices used for downstream analysis. Specifically, data from three WT and three Rptor1⁻/⁻Cre mice were combined into one data set for consistent filtering, and UMIs mapped to genes encoding ribosomal proteins were removed. Cells with low UMI counts (potentially dead cells with broken membranes) or high UMI counts (potentially two or more cells in a single droplet) were filtered. A small fraction of outlier cells (430) was further removed because of their low transcriptome diversity (meaning that fewer genes were detected than in other cells with a comparable number of captured UMIs). A total of 27,619 cells (WT, 13,295; Rptor1⁻/⁻Cre, 14,324) was captured, with an average of 3,419 messenger RNA (mRNA) transcript counts (UMIs, mean±SD range: 1,500–9,899). We normalized the expression level of each gene to 10,000 UMIs per cell and log-transformed them by adding 0.5 to the expression matrix.

Clustering. We inferred the subpopulation structure of the whole data set by using linear latent cell state analysis (LCA)41, a clustering algorithm developed in house for analysing large-scale scRNA-seq data (C. Cheng et al., manuscript submitted). Briefly, LCA first used singular-value decomposition (SVD) to derive latent cellular states from the expression matrix for individual cells. Significant cellular states were determined using the Tracy–Widom test on eigenvalues. A modified version of spectral clustering was performed on the significant cellular states of individual cells (cellular states were explained by total UMIs ignored) with different numbers of clusters (2–30). The optimal number of clusters was manually selected from top models determined by the silhouette measure for solutions with different numbers of clusters.

Data visualization. Underlying cell variations derived from WT and Rptor1⁻/⁻Cre single-cell gene expression were visualized in a two-dimensional projection by t-distributed stochastic neighbour embedding (tSNE). Expression of individual genes or pathway scores was colour-coded (grey, not expressed; from low to high, blue–green–yellow–red) for each cell on tSNE plots. Generation of gene signatures and statistical analysis for functional enrichment. We used published gene sets that are shared between memory and T17 cells ('memory T17 overlap UP' and 'memory T17 overlap DOWN' for upregulated and downregulated genes, respectively)31, and early and late CD8' T cell memory gene sets used previously to show memory features of T17 cells ('early memory' and 'late memory')32. We generated a Tbx21 (T-bet)-dependent signature ('T-bet targets') from previously published gene-expression changes in cultured T17 cells sufficient or deficient for Tbx21 (log2 fold change ≥ 3; GSE38808)33. A microarray data set was used as the APM for customer-facing annotation and the package R to generate 'CD253fa effector CD8' and 'CD253d memory-precursor CD8' gene signatures. Specifically, upregulated and downregulated genes in the CD253 versus CD253d comparison were ranked after filtering at 5% FDR by log fold change, and 196 upregulated
genes (with a log fold change greater than 1) were annotated as CD25+ effector CD8, and the top 200 downregulated genes (with a log fold change of less than −1) were annotated as CD25− memory-precursor CD8. Similarly, we used a microarray data set (GSE84105) to generate CXCR5+-exhausted CD8 (Ahmed)’ and CXCR5− exhausted CD8 (Ahmed)’ gene signatures at the same significance level of 5% FDR; total upregulated and downregulated genes were more than 200, so we ranked genes by their log fold change of expression in the CXCR5+ versus CXCR5− comparison, and used the top 200 upregulated genes as ‘CXCR5+ exhausted CD8 (Ahmed)’ and the top 200 downregulated genes as ‘CXCR5− exhausted CD8 (Ahmed)’. Furthermore, we processed RNA-seq data (GSE76279) using the DEseq2 R package version 1.16.1 to generate ‘CXCR5+ exhausted CD8 (Yu)’ and ‘CXCR5− exhausted CD8 (Yu)’ gene signatures via the same strategy as above. For scRNA-seq analysis, we retained genes from these signatures if they were expressed in at least 10% of cells in any cluster. Gene-signature activity was calculated as the average value for all retained genes. For functional enrichment of genes highly expressed in a specific cluster, we applied the Fisher’s exact test (R version 3.3.1) using the specific gene signatures described above, or hallmark, canonical pathways and gene-ontology gene sets (MSigDB)11.

Pseudotime analysis using Monocle 2. Pseudotime analysis was performed using Monocle 226 (v. 2.3.6) with cell ordering based on highly dispersed and highly expressed genes (empirical dispersion/dispersion fit ≥ 1.1 and mean expression ≥ 0.01; Extended Data Fig. 10a). Resulting data were visualized using functions available in the Monocle 2 package and ggplot2 (v. 2.2.1).

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

Data availability
Microarray data are available via GEO (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE107521. ATAC-seq and scRNA-seq data are available via GEO under accession number GSE121599.

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Extended Data Fig. 1 | CD27 expression on T_{H}17 cells during autoimmunity, and cellular homeostasis of CD27^{+} and CD27^{-} T_{H}17 subsets. a, Analysis of publicly available single-cell transcriptomics data from IL-17–GFP^{+} cells in dLN compared with the CNS. b, Flow cytometry analysis of putative memory or activation markers on CD4^{+} TCR-β^{+} YFP^{+} cells from the dLN and spleen of mice at day nine post MOG-immunization. c, Summary of CD27 expression on CD4^{+} TCR-β^{+} YFP^{+} cells in dLN (red; n = 5, day 5; n = 3, day 7; n = 7, day 9; n = 8, day 16) overlaid with clinical EAE score (black, n = 5). d, Ki-67 expression in CD27^{+} and CD27^{-} cell populations. e, Flow cytometry analysis (left) and summary (right) of CD27 expression on transferred CD27^{+} or CD27^{-} YFP^{+} cells, at day 14 after transfer into CD45.1^{+} hosts (n = 14, CD27^{+}; n = 15, CD27^{-}). f, Flow cytometry analysis of Bcl-2 expression in CD27^{+} and CD27^{-} populations. Data are means ± s.e.m. and representative of three (b–d, f) or at least five (e) independent experiments. Numbers in plots represent frequencies of cells in gates; numbers within histograms represent mean fluorescence intensities. The Mann–Whitney U-test (two-sided; non-parametric) was used in e to determine statistical significance.
Extended Data Fig. 2 | Gene-expression profiles associated with CD27+ and CD27− T<sub>H</sub>17 subsets. a, Volcano plot of transcriptomics data in CD27+ versus CD27− CD4+ TCR-β+ YFP+ cells. b, GSEA plots comparing CD27+ and CD27− populations using gene sets of antigen-specific CXCR5+ and CXCR5− exhausted CD8+ T cells from chronic infection. Gene-expression heat maps are normalized by row (z-score) for the top 30 leading-edge genes between microarray samples from CD27+ versus CD27− CD4+ TCR-β+ YFP+ cells, using gene sets derived from CXCR5+ and CXCR5− exhausted CD8+ T cells. c, Gene-expression heat maps normalized by row (z-score) for the top 30 leading-edge genes in CD27+ versus CD27− CD4+ TCR-β+ YFP+ cells, using the apoptosis hallmark gene set. d, GSEA plots comparing CD27+ and CD27− populations using hallmark gene sets, showing the enrichment of mTORC1 signalling, Myc targets, and selective metabolic pathways in CD27− T<sub>H</sub>17 cells. Data are from one experiment (a–d). NES, normalized enrichment score.
Extended Data Fig. 3 | Cell-intrinsic requirement for Raptor in Th17 cells. a, Naive CD4\(^{+}\) T cells were differentiated under Th17-polarizing conditions and analysed for cytokine expression after PMA/ionomycin stimulation in vitro (left), or for proliferation (CellTrace Violet) and ROR-\(\gamma\) expression (right). b, Flow cytometry analysis (left) and total number of CD4\(^{+}\) T cells (right) from spinal cord at day 16 post-immunization (\(n=10\), WT; \(n=8\), Rptor\(^{Il17aCre}\)). c, Experimental design for the generation (left) and clinical scores (right) of WT and Rptor\(^{Il17aCre}\) (R26ReYFP) bone-marrow (BM) chimaeras for restriction of Raptor deficiency specifically to TCR-\(\alpha\)-expressing IL-17\(^{+}\) T cells (\(n=5\) per genotype). Specifically, a 5/1 ratio of Tcra\(^{-/-}\) and Rptor\(^{Il17aCre}\) (or WT control) BM cells was transferred into sublethally irradiated Tcra\(^{-/-}\)/− recipients, followed by reconstitution. In this system, all T cells in the chimaeras were derived from Rptor\(^{Il17aCre}\) or WT BM cells, whereas most non-T-cell compartments were derived from WT cells. d, Experimental design for the generation (left) and clinical scores (right) of WT and Rptor\(^{Il17aCre}\) (R26ReYFP) BM chimaeras for equal inflammatory conditions. Specifically, mixed BM chimaeras were generated using a 1/1 ratio of congenically marked CD45.2\(^{+}\) Rptor\(^{Il17aCre}\) (or WT control) and CD45.1\(^{+}\) WT BM-derived cells, which mediated CNS inflammation in EAE (\(n=3\), WT; \(n=4\), Rptor\(^{Il17aCre}\)). e–k, The equi-inflammatory chimaeric mice generated in panel d were analysed at day 18 post-MOG immunization, for the frequencies of CD4\(^{+}\) T cells positive for CD45.2 or YFP (e; \(n=12\), WT; \(n=14\), Rptor\(^{Il17aCre}\)) and YFP\(^{+}\) CD4\(^{+}\) T cells expressing IL-17 or IFN-\(\gamma\) within the spinal cord (f; \(n=6\), WT; \(n=7\), Rptor\(^{Il17aCre}\)); the expression of Ki-67 (g; \(n=8\), WT; \(n=10\), Rptor\(^{Il17aCre}\)), T-bet (h; as fold-change in mean fluorescence intensity after normalization to WT cells) (\(n=10\) per genotype), and active caspase-3 in splenic YFP\(^{+}\)CD4\(^{+}\) T cells (i; \(n=6\) per genotype); and the expression of CCR6 (j; \(n=7\), WT; \(n=6\), Rptor\(^{Il17aCre}\)) and CXCR3 (k; \(n=8\), WT; \(n=10\), Rptor\(^{Il17aCre}\)) in YFP\(^{+}\) cells from different organs. Data are means ± s.e.m. and representative of three (a, c, i–k) or four (b, d–h) independent experiments. Numbers in plots represent frequencies of cells in gates or quadrants. Student's t-test (two-sided) was used in h and k, and Mann–Whitney U-test (two-sided) was used in b, e, f and g, to determine statistical significance.
Extended Data Fig. 4  |  Raptor deficiency induces selective phenotypic changes in fate-mapped Th17 cells. a–g, WT and Rptor$^{Il17aCre}$ (R26R$^{YFP}$) mice were immunized with MOG, and YFP$^+$ cells from dLN were analysed at day nine post-immunization. a, Frequency of YFP$^+$ cells ($n = 7$ per genotype). b, Efficiency of Rptor deletion (left; $n = 7$ per genotype) and flow cytometry analysis of phosphorylated S6 (S235/236) and 4E-BP1 (T37/46) (right) in YFP$^+$ cells. c, Flow cytometry analysis of active caspase-3 and 7-AAD staining in YFP$^+$ cells. d, Flow cytometry analysis of CXCR3 and CCR6 expression on YFP$^+$ cells. e, Cytokine production by YFP$^+$ cells from dLN ($n = 7$ per genotype). f, Real-time PCR analysis of Tbx21 ($n = 8$ per genotype), Rorc ($n = 6$ per genotype), Il12rb2 ($n = 7$ per genotype) and Il23r ($n = 7$ per genotype) expression in YFP$^+$ cells. g, Flow cytometry analysis of Foxp3 expression. h, i, Cytokine production (h, i) and proliferation (h) of YFP$^+$ cells from dLN of the indicated mice after four days of stimulation with MOG alone (h) or with MOG plus IL-23 (i) ($n = 7$ per genotype). j, Sorted YFP$^+$ cells were stimulated with IL-23 or IL-12 for 30 min in vitro and stained with specific antibodies to phosphorylated STAT3 (left), phosphorylated STAT4 (right), or isotype controls. Data are means ± s.e.m. and representative of seven (a), three (b–f), two (g), or five (h, i) independent experiments. Numbers in plots represent frequencies of cells in quadrants; numbers within histograms represent mean fluorescence intensities. Student's $t$-test (two-sided) was used in b, and Mann–Whitney U-test (two-sided) in a, e, i, to determine statistical significance.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Altered gene-expression profiles in Raptor-deficient cells, and shared functional pathways with CD27+ T H 17 cells. a–d, WT and Rptor<sup>Il17aCre<sup> (R26R<sup>YFP</sup>) mice were immunized with MOG, and sorted YFP+ cells from dLN were analysed by microarray. a, Expression of individual genes, with vertical lines indicating −1.5-fold and +1.5-fold cut-offs and a horizontal line indicating the P = 0.05 cut-off; gene-ontology (GO) gene sets for acetylation and cholesterol biosynthesis are coloured and Hmgcr is indicated. b, GSEA reveals significant ‘hallmark’ gene sets that are downregulated in Rptor<sup>Il17aCre</sup> compared with WT cells (P < 0.05). c, d, Comparison of functional enrichment of coregulated gene sets in Rptor<sup>Il17aCre</sup> and CD27+ YFP+ cells. We used the downregulated genes in Rptor<sup>Il17aCre</sup> versus CD27+ T<sub>H</sub>17 cells and in Rptor<sup>Il17aCre</sup> versus WT T<sub>H</sub>17 cells (with an FDR of less than 0.05 and the top 200 genes, based on fold change) for functional enrichment using hallmark (c) and KEGG pathway (d) gene sets. Keys indicate FDR values and enrichment scores. e, Comparison of microarray analyses of CD27+ versus CD27− and Rptor<sup>Il17aCre</sup> versus WT samples from mice at day nine post MOG-immunization. Shown are empirical cumulative distribution functions for the changes in expression (log<sub>2</sub> values) of all genes expressed in Rptor<sup>Il17aCre</sup> (R26R<sup>YFP</sup>) T<sub>H</sub>17 cells (red line; changes are relative to expression in WT T<sub>H</sub>17 cells) and for subsets of genes downregulated (green line) or upregulated (blue line) in CD27+ versus CD27− T<sub>H</sub>17 cells (with a FDR of less than 5%). P-values were calculated using the Kolmogorov–Smirnov test. f, Real-time PCR analysis of Cd27 expression in WT and Rptor<sup>Il17aCre</sup> YFP+ cells (n = 5 per genotype). Data are means ± s.e.m. and from one experiment (a–e) or representative of two independent experiments (f). Student’s t-test (two-sided) was used in f to determine statistical significance.
Extended Data Fig. 6 | Anabolic metabolism promotes the transdifferentiation of Th17 cells into Th1-like IFN-γ-producing cells. a, Ingenuity pathway analysis (IPA) of upstream transcriptional regulators in Rptor$^{Il17aCre}$ versus WT samples. b, WT and Rptor$^{Il17aCre}$ (R26R$^{YFP}$) mice were immunized with MOG, and YFP$^+$ cells from dLN were analysed by flow cytometry for intracellular expression of Myc. c, d, WT and Myc$^{Il17aCre}$ (R26R$^{YFP}$) mice were immunized with MOG, and YFP$^+$ cells from dLN were analysed by real-time PCR at day nine (c; n = 4, Tbx21; n = 2, Rorc; n = 4, Il12rb2; n = 4, Il23r), or alternatively dLN cells were cultured with MOG, MOG plus IL-12, or MOG plus IL-23 for four days in order to analyse cytokine expression within YFP$^+$ cells (d). e, f, WT and Hmgcr$^{Il17aCre}$ (R26R$^{YFP}$) mice were immunized with MOG; YFP$^+$ cells were isolated from dLN and analysed by real-time PCR (e; n = 4, Tbx21; n = 2, Rorc; n = 4, Il12rb2; n = 4, Il23r), or alternatively dLN cells were cultured with MOG, MOG plus IL-12, or MOG plus IL-23 for four days in order to analyse cytokine expression within YFP$^+$ cells (f). g, Flow cytometry analysis of CD27 expression on YFP$^+$ cells from WT, Rptor$^{Il17aCre}$ Myc$^{Il17aCre}$ and Hmgcr$^{Il17aCre}$ mice at day nine post MOG-immunization. h, i, WT (R26R$^{YFP}$) mice were immunized with MOG, and dLN cells were stimulated with MOG and IL-12 in the presence of vehicle, PF-4708671 (an inhibitor of S6K phosphorylation) or Cbz-B3A (an inhibitor of 4E-BP phosphorylation, which in turn suppresses eIF4E-dependent protein translation) at the indicated concentrations for four days to analyse cytokine expression within YFP$^+$ cells (h; right, summary plots) (n = 9, vehicle; n = 7, PF-4708671 (5 μM); n = 9, PF-4708671 (10 μM); n = 7, Cbz-B3A (5 μM); n = 9, Cbz-B3A (10 μM)) and for CellTrace Violet dilution (i). Data are means ± s.e.m. and from one experiment (a), or representative of four (b-f, h, i) or three (g) independent experiments. Numbers in plots represent frequencies of cells in gates or quadrants; numbers within histograms represent mean fluorescence intensities. Student’s t-test (two-sided; parametric) was used to determine statistical significance in panel h.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Analysis of histone acetylation, and ATAC-seq overview and specific gene loci. a, ChIP qRT–PCR of pan-acetyl-histone bound to the *Ifng* promoter of WT or *Rptor*^{Il17aCre}^{R26R^{YFP}} YFP− cells from dLN (n = 4 per genotype). b, c, WT and *Rptor*^{Il17aCre}^{R26R^{YFP}} mice were immunized with MOG, and YFP+ cells from dLN and spleen at day nine post-immunization were analysed by ATAC-seq. b, Density plot and heat maps of a representative individual ATAC-seq sample, demonstrating separation into different fragment lengths indicative of nucleosome-free, mononucleosome, dinucleosome and trinucleosome patterns, consistent with ref. 15. TSS, transcription start site. c, Correlation plot of nucleosome-free fragments. d, Nucleosome-free ATAC-seq tracks at the *Tbx21* and *Il12rb2* gene loci, with immediate promoter regions indicated by red boxes. e, Summary of ATAC-seq motif-enrichment data, showing log_{2} (odds ratio) and log_{10} (Fisher *P*-value) of cells from dLN. f, Tn5 insert sites from ATAC-seq analysis of dLN were aligned to motifs for transcription factors from the TRANSFAC database, and the binding profiles of selected transcription factors of the TCF–LEF family are shown. Data are means ± s.e.m. and representative of three independent experiments (a), or from one experiment (b–f). Student’s *t*-test (two-sided; parametric) was used in panel a to determine statistical significance.
Extended Data Fig. 8 | ATAC-seq in-depth analyses, TCF-1 binding activity, and effects of 2-DG on cytokine expression. a, Analysis of common regions in ATAC-seq and ChIP-seq peaks for motifs that bind to TCF–LEF family and T-bet transcription factors, from spleen samples. Numbers of motif matches and associated Fisher’s exact test $P$-values and log2 (odds ratios) are shown; a positive log2 (odds ratio) value indicates that a motif is more likely to occur in \textit{Rptor}^{Il17aCre} than in WT samples; a negative value indicates that the chance of occurrence is lower in the \textit{Rptor}^{Il17aCre} group. 'E $\times 10^{-x}'$ denotes '$\times 10^{-x}$'. b, Nucleosome-free ATAC-seq tracks at the Il6ra and Lrig1 gene loci, with TCF-1-binding sites indicated by red boxes, based on alignment with TCF-1-binding sites from published data (GEO accession numbers are shown). c, ChIP assay to measure TCF-1 binding to Il6ra and Lrig1 gene loci (Il6ra, n = 2 per genotype; Lrig1, n = 6 for WT, n = 5 for \textit{Rptor}^{Il17aCre}). d, Cells from dLN of the indicated mice at day nine post-MOG immunization were cultured for four days with MOG plus IL-12 and sorted on the YFP$^+$ population before intracellular staining. Flow cytometry was used to analyse T-bet and TCF-1 expression in YFP$^+$ cells from WT and \textit{Myc}^{Il17aCre} (R26R$^{EYFP}$) mice. e, Tn5 insert sites from ATAC-seq analysis of YFP$^+$ cells from PBS- or 2-DG-treated mice were aligned to motifs for transcription factors from the TRANSFAC database, and the binding profiles of selected TCF–LEF family transcription factors are shown. f, Cytokine expression in dLN YFP$^+$ cells from MOG-immunized Il17aCre (R26R$^{EYFP}$) mice after culture with MOG and IL-12 for four days in the presence of vehicle (PBS) or 2-DG (1 mM). g, Cytokine expression in splenic YFP$^+$ cells from MOG-immunized Il17aCre (R26R$^{EYFP}$) mice after treatment with 2-DG (2 g per kg of body weight) or PBS. Numbers in plots represent frequencies of cells in gates or quadrants. Data are means ± s.e.m. and from one experiment (a, b, e), or representative of three independent experiments (c, d, f, g). Student's $t$-test (two-sided) was used to determine statistical significance in c.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Single-cell transcriptomics analysis. WT and Rptor\(^{R172C_{Cre}}\) (R26\(^{R_{YFP}}\)) mice were immunized with MOG, and YFP\(^{+}\) cells were analysed by single-cell transcriptomics at day nine post-immunization. a, Membership of individual cell clusters in two-dimensional tSNE projections from scRNA-seq data. b, tSNE visualization of nine clusters partitioned by unsupervised clustering. c, Frequencies of WT and Rptor\(^{R172C_{Cre}}\) cells in different clusters (\(n = 3\) per genotype). d, Top three enriched gene sets for each of the clusters using 'hallmark', 'canonical' and GO gene sets. For example, genes enriched in cluster 1 by comparison with other clusters were associated with proliferative events. e, Summary of cluster-specific functional enrichment analysis via Fisher’s exact test, using the signatures of 'T-bet targets,' 'late memory,' 'memory T\(_{FH}\) overlap DOWN,' 'HALLMARK_GLYCOLYSIS,' 'memory T\(_{FH}\) overlap UP' and 'early memory' as described in the Methods. f, tSNE visualization of signature scores of 'T-bet targets' and 'HALLMARK_GLYCOLYSIS' expressed in individual cells. g, Violin plots of \(Bc12\), \(Cd27\) and \(Tcf7\) gene expression amongst the nine clusters. A violin plot combines the box plot and the local density estimation into a single display. The black bars and thin lines within the violin plots indicate, respectively, the interquartile range (first quantile to third quantile) and the entire range of the data (up to 1.5-fold of the interquartile range from first to third quantile); the white dots in the centre indicate the median values. Data are from one experiment (a–g) (\(n = 3\) per genotype).
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Pseudotime analysis and experimental validation. a–h, WT and Rpto\textsuperscript{Il17aCre} (R26R\textsuperscript{YFP}) mice were immunized with MOG, and YFP\textsuperscript{+} cells were analysed by single-cell transcriptomics analysis at day nine post-immunization. a, Empirical dispersion and mean expression using the single-cell-analysis toolkit Monocle 2, including the genes used for temporal ordering in black; each grey or black dot represents one gene. The red line shows Monocle’s expected dispersion, with more- and less-dispersed genes based on average expression above and below the red line, respectively. b, Pseudotime densities for each individual cluster. For example, cluster 1, associated with the proliferative signature, was in the centre of the pseudotime spectrum, while clusters 2, 3 and 8 (early in pseudotime; predominantly Raptor-deficient cells) and clusters 7 and, to a lesser extent, 4 and 5 (late in pseudotime; predominantly WT cells) were on the opposite end of the spectrum. c, Projection of signature scores for 'early memory', 'late memory' and 'T-bet targets' onto pseudotime trajectory; the keys indicate the relative scores per cell. d, tSNE visualization of Tbx21 and Ifng gene expression. e, Tbx21 and Ifng gene expression during pseudotime; cells that did not express Tbx21 or Ifng were filtered out in their respective graphs. f, Pseudotime assignment for WT and Rpto\textsuperscript{Il17aCre} cells, coloured by genotype; each dot represents one cell. g, Cd27 and Tcf7 gene expression across pseudotime, coloured by genotype. h, tSNE visualization of Cd27 and Tcf7 expression. i, Flow cytometry analysis of T-bet expression in freshly isolated CD27\textsuperscript{+} and CD27\textsuperscript{-} cells from dLN of Il17aCre (R26R\textsuperscript{YFP}) mice at day nine post MOG-immunization. j, Fold change in the percentage of the IL-17\textsuperscript{-} IFN-\gamma\textsuperscript{+} cells amongst CD27\textsuperscript{+} or CD27\textsuperscript{-} YFP\textsuperscript{+} cells stimulated with MOG plus IL-12 as compared with freshly isolated cells (n = 12). k, CD27\textsuperscript{+} YFP\textsuperscript{+} cells from MOG-immunized WT and Rpto\textsuperscript{Il17aCre} mice were sorted and transferred into CD45.1\textsuperscript{+} hosts. The following day, CD45.1\textsuperscript{+} host mice were immunized with MOG; four days later, YFP\textsuperscript{+} cells were analysed by flow cytometry for surface CD27 expression (left; a summary plot is at the right: n = 6, WT; n = 5, Rpto\textsuperscript{Il17aCre}). l, CD27\textsuperscript{+} YFP\textsuperscript{+} cells from MOG-immunized WT and Rpto\textsuperscript{Il17aCre} mice were stimulated with MOG plus IL-12 for four days, and then CD27 expression was analysed. m, \(T_{H17}\) cells are functionally and metabolically heterogeneous, and are composed of a subset with stemness features but lower anabolic metabolism, and a reciprocal subset with higher metabolic activity that supports transdifferentiation into \(T_{H1}\) cells. These two subsets are further distinguished by selective expression of the transcription factors TCF-1 and T-bet, respectively, and discrete levels of CD27 expression. mTORC1 activation drives reprogramming of anabolic metabolism, favouring transcription that is mediated by T-bet rather than TCF-1; consequently, \(T_{H17}\) transdifferentiation into \(T_{H1}\)-like \(T_{H17}\) cells occurs. Memory/stem-like \(T_{H17}\) cells can become reactivated and have the potential to undergo terminal differentiation and acquire \(T_{H1}\)-like phenotypes. Data are means ± s.e.m. and from one experiment (a–h), or are representative of three (i) or five (j–l) independent experiments. Numbers in plots represent frequencies of cells in gates; numbers within histograms represent mean fluorescence intensities. Mann–Whitney U-test (two-sided) was used in panel j to determine statistical significance.
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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| Item                                                                 | Status |
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| The statistical test(s) used AND whether they are one- or two-sided | Confirmed |
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| State explicitly what error bars represent (e.g. SD, SE, CI) | Confirmed |

Software and code

Policy information about availability of computer code

Data collection

The following software was used for data collection: BD FACSDIVA™ software for FACS, Affymetrix GeneChip Command Console Scan Control software v 4.0.0.1567 for microarray, HiSeq Control Software 2.0.12.0 and RTA version 1.17.21.3 for Sequencing data.

Data analysis

Methods describe the software used to analyze microarray, ATAC-Seq, and scRNA-Seq samples in detail and are publicly available unless otherwise stated. Latent Cellular State Analysis (LCA), a novel clustering algorithm developed in house for analyzing large-scale scRNA-Seq data (manuscript in preparation). Flowjo 9.3.2 or 9.9.6 (Tree Star) for FACS results; GraphPad Prism 6 for statistics.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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Transcriptome data have been deposited into NCBI GEO (GSE107521 and GSE121599).

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Life sciences

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Life sciences study design

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

Sample size: Sample size was selected to maximize the chance of uncovering mean difference which is also statistically significant.

Data exclusions: No data were excluded.

Replication: Each experiment used a new cohort of mice with very consistent results. All replicates reported in the manuscript are biological replicates. All the statistics reported in the manuscript are based on at least 3 biologically independent replicates.

Randomization: Age- and sex-matched mice were assigned randomly to experimental and control groups.

Blinding: The assessment of EAE scores and histopathology examination was performed in a blinded fashion. Experimental analyses of mouse samples were obtained by automated methods (cell counter, flow cytometer, realtime PCR machine, et al.). Other experimental techniques were not blinded.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a Involved in the study

- Unique biological materials
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants

Methods

n/a Involved in the study

- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

Antibodies used: The following antibodies were used: anti-CD27 (LG.7F9), anti-CD45.2 (104), anti-ifNγ (XMG1.2), anti-KLRG1 (2F1), anti-T-bet (eBio4B10) (all from eBioscience); anti-CD43 (activation-glycoform; 1B11), anti-CD44 (IM7), anti-CD45.1 (A20), anti-CD62L (MEL-14), anti-CD127 (A7R34), anti-CCR6 (29-2L17), anti-CXCR3 (OXCR3-173), anti-Il-17 (TC11-18H10.1), anti-Ly6C (HK1.4), anti-PO-1 (29F.1A12), anti-Sca-1 (D7), anti-TCRb (H57-597) (all from Biolegend); anti-CD95 (Jo2), anti-Foxp3 (FJK-16s), anti-RORγt (O31-378), anti-pSTAT3 (pY705), anti-pSTAT4 (pY693) (all from BD Biosciences); and anti-CD4 (RM4-5) (from SONY), anti-p4E-BP1 T37/46 (236B4), anti-pS6 S235/236 (D57.2.2E), and anti-TCF-1 (C63D9) (from Cell Signaling Technology) (from Methods section).

Validation

Antibodies were validated by the manufacturer and corroborated with previously observed antibody staining results for FACS.
Animals and other organisms

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

Laboratory animals

Mice were housed and bred at the St. Jude Children’s Research Hospital animal care facilities in specific pathogen-free conditions. C57BL/6, TCRα−/−, CD45.1+, Il17aCre, Rag1−/− and 2D2-transgenic mice were purchased from the Jackson Laboratory. Rptorfl, Mycfl, and Hmgcrfl mice were as described previously22-24. Cre-expressing mice were used as controls, and littermates were used when possible. Mice were backcrossed for at least 10 generations to the C57BL/6 background strain. Female and male mice were used at 6-10 weeks of age (from Methods section).

Wild animals

No wild animals were used in the study.

Field-collected samples

No field-collected samples were used in the study.

Flow Cytometry

Plots

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

Methodology

Sample preparation

Spleens, peripheral draining lymph nodes (dLN), and spinal cord (SC) samples were gently ground under nylon mesh using the flat end of a 3-mL syringes. Red blood cells were removed by ACK lysing buffer, followed by washing cells with isolation buffer. Cells were then filtered, pelleted and stained for FACS.

Instrument

BD LSR II and LSR-Fortessa.

Software

FACSDIVA 7.0+ for sample collection. FlowJo 9.3.2 or higher or FlowJo 10 (Tree Star) for FACS analysis.

Cell population abundance

The purities of the sorted T cells were more than 99%.

Gating strategy

Based on the pattern of FSC-A/SSC-A, cells in the lymphocyte gate were used for analysis of T cell subsets. Singlets were gated according to the pattern of FSC-H vs. FSC-A. Positive populations were determined by the specific antibodies, which were distinct from negative populations. An example gating strategy is shown in supplementary information.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.